Steroidogenesis-Inducing Protein: An enigmatic protein with multiple biological functions

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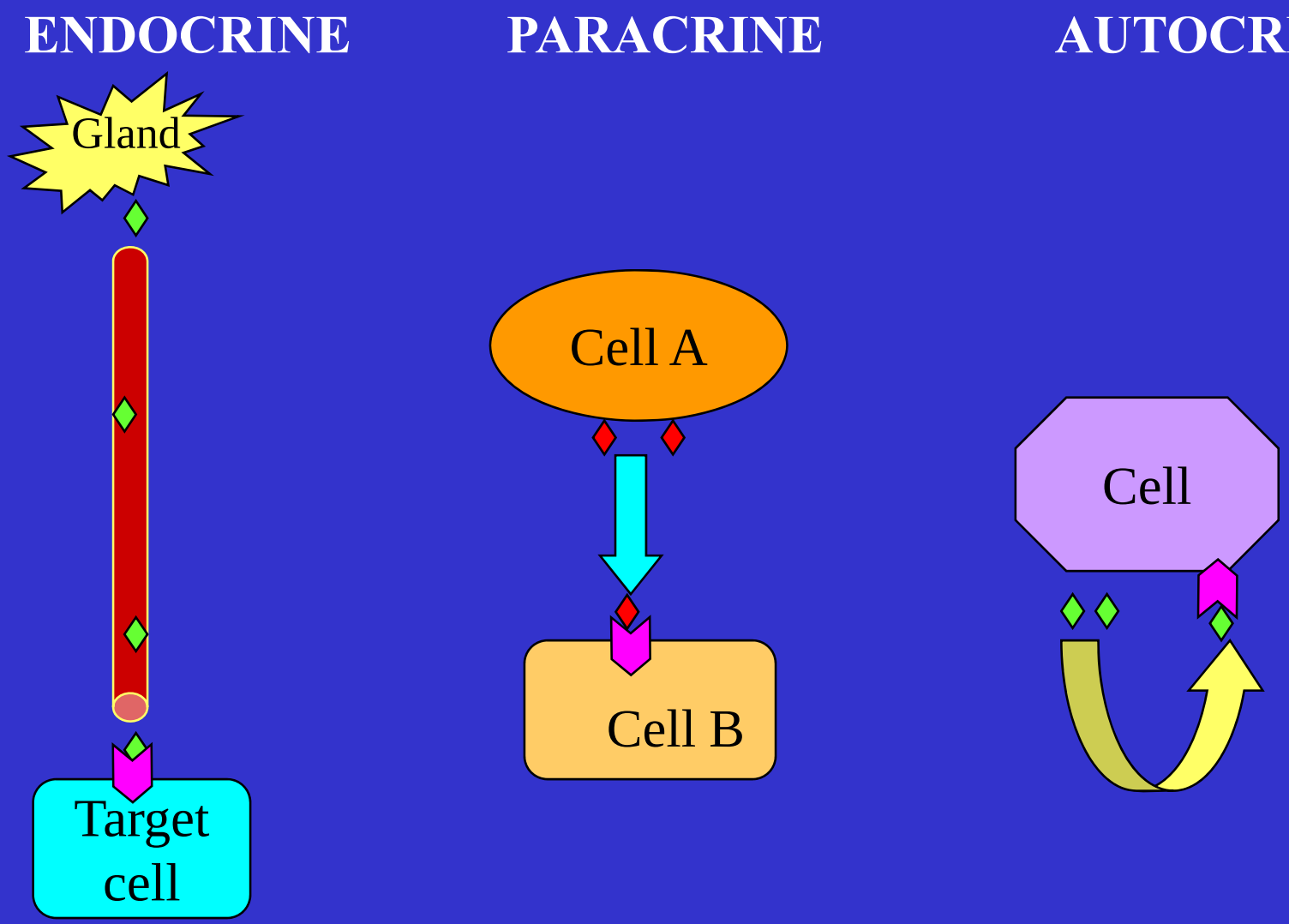
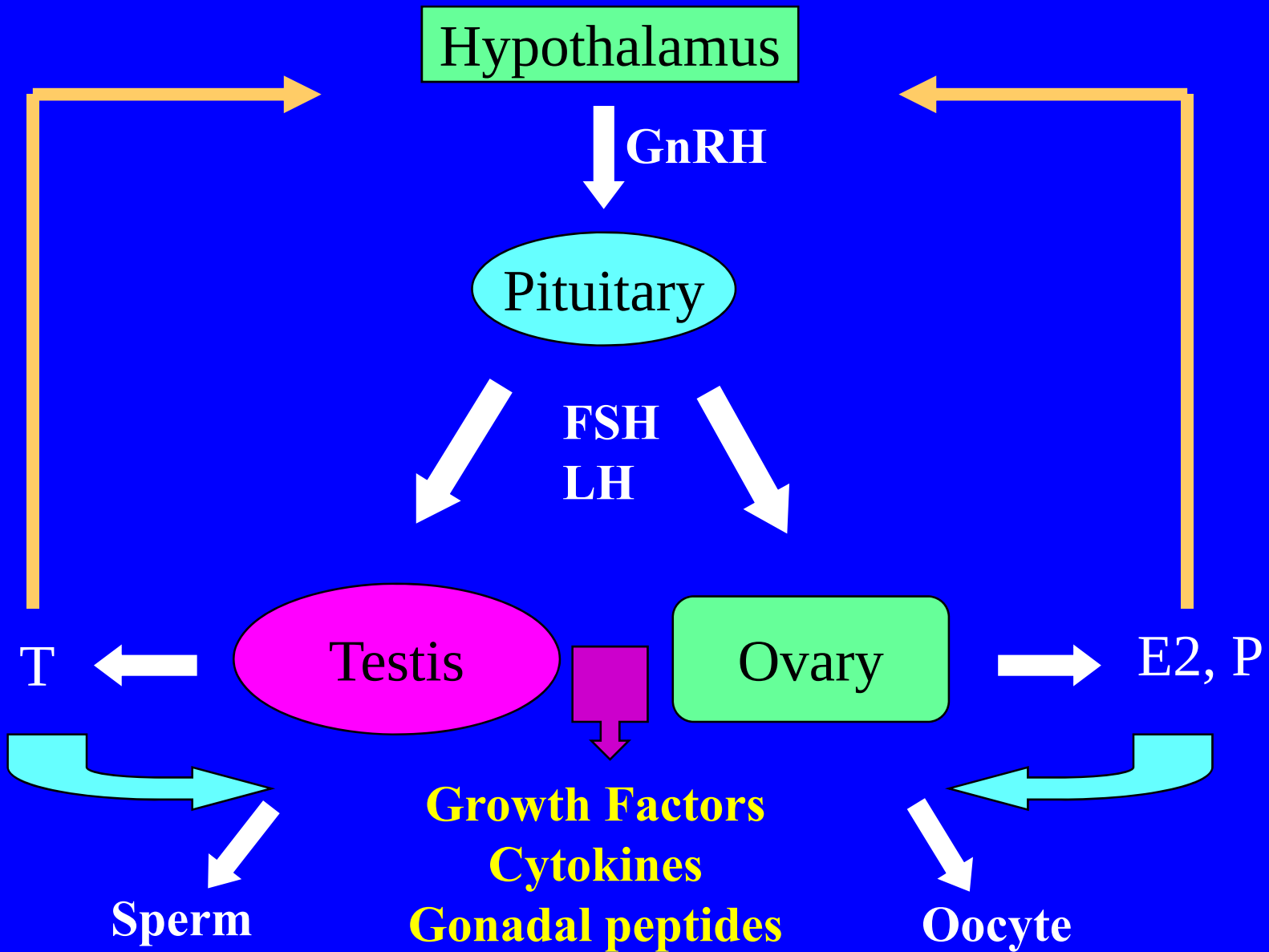
Research Projects

- TGFβ in prostate cancer: intracellular signaling mechanisms involved in TGFβ effects on proliferation, invasion and metastasis of prostate cancer cells.
- Mechanisms involved in development of androgen independence in advanced stages of prostate cancer.
- Role of SIP in prostate cancer.

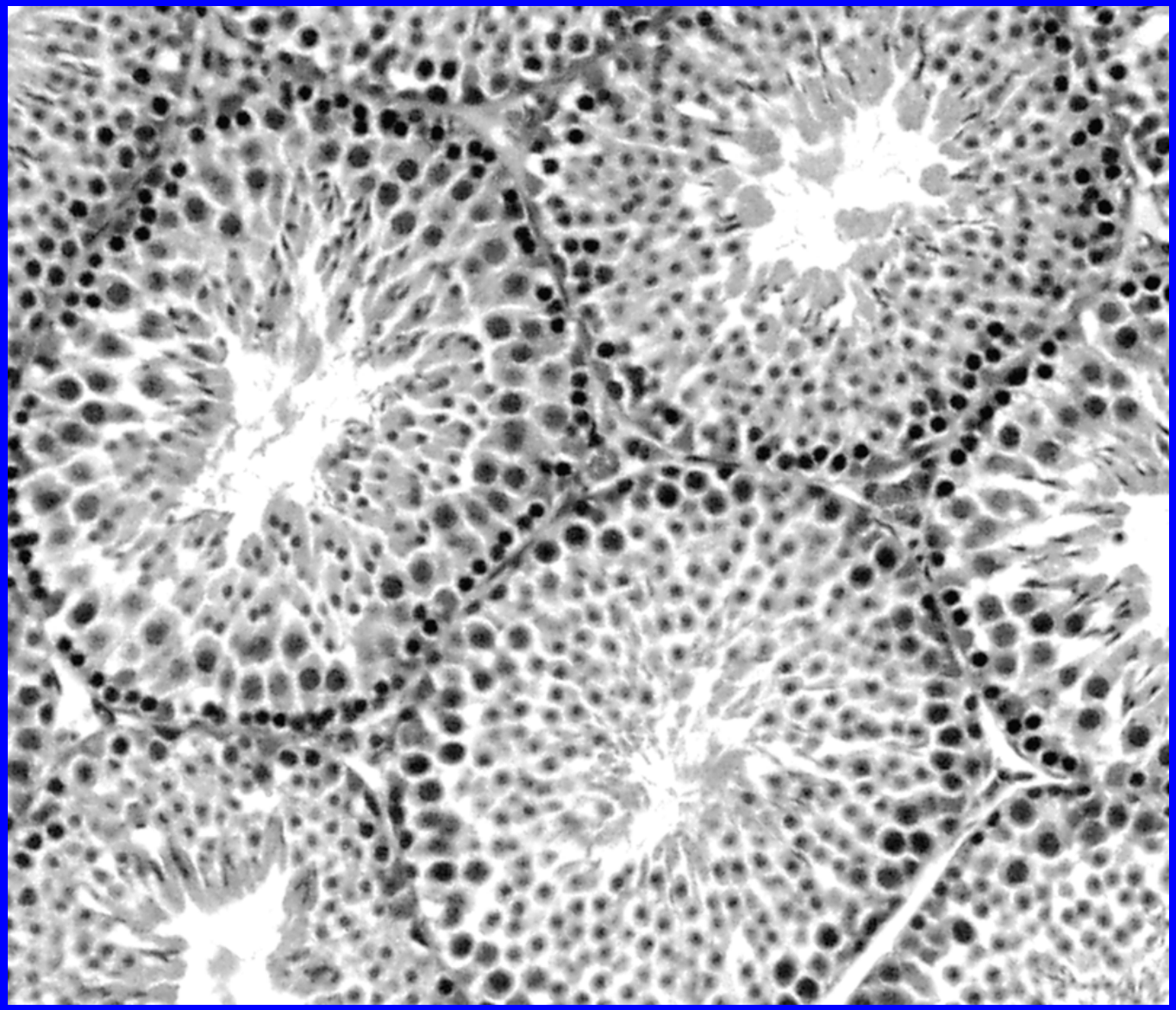
Integration of Biological Functions by Chemical Messengers



Regulation of Reproductive Development and Function



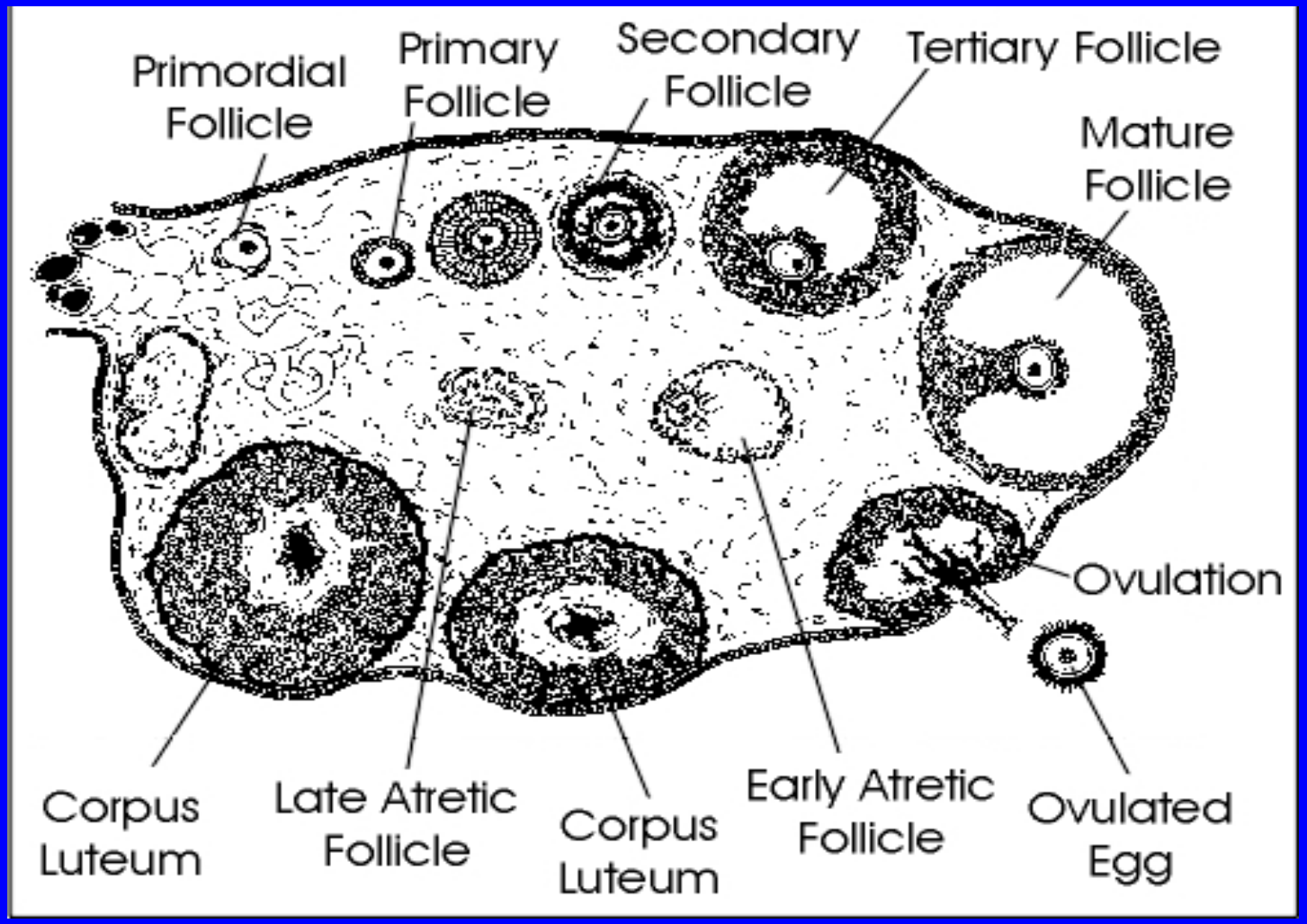
Paracrine and Autocrine Regulation of Testis



Experimental Approaches

1. **Effects of known growth factors and cytokines on testicular cells.**
2. **Identification of novel bioactive peptides and proteins in testicular secretions.**

Follicular Development in the Human Ovary



Follicular Fluid Proteins

1. Steroidogenesis
2. Proliferation
3. Luteinization and Luteal Function

Effects of hFF on Steroidogenesis in Leydig Cells

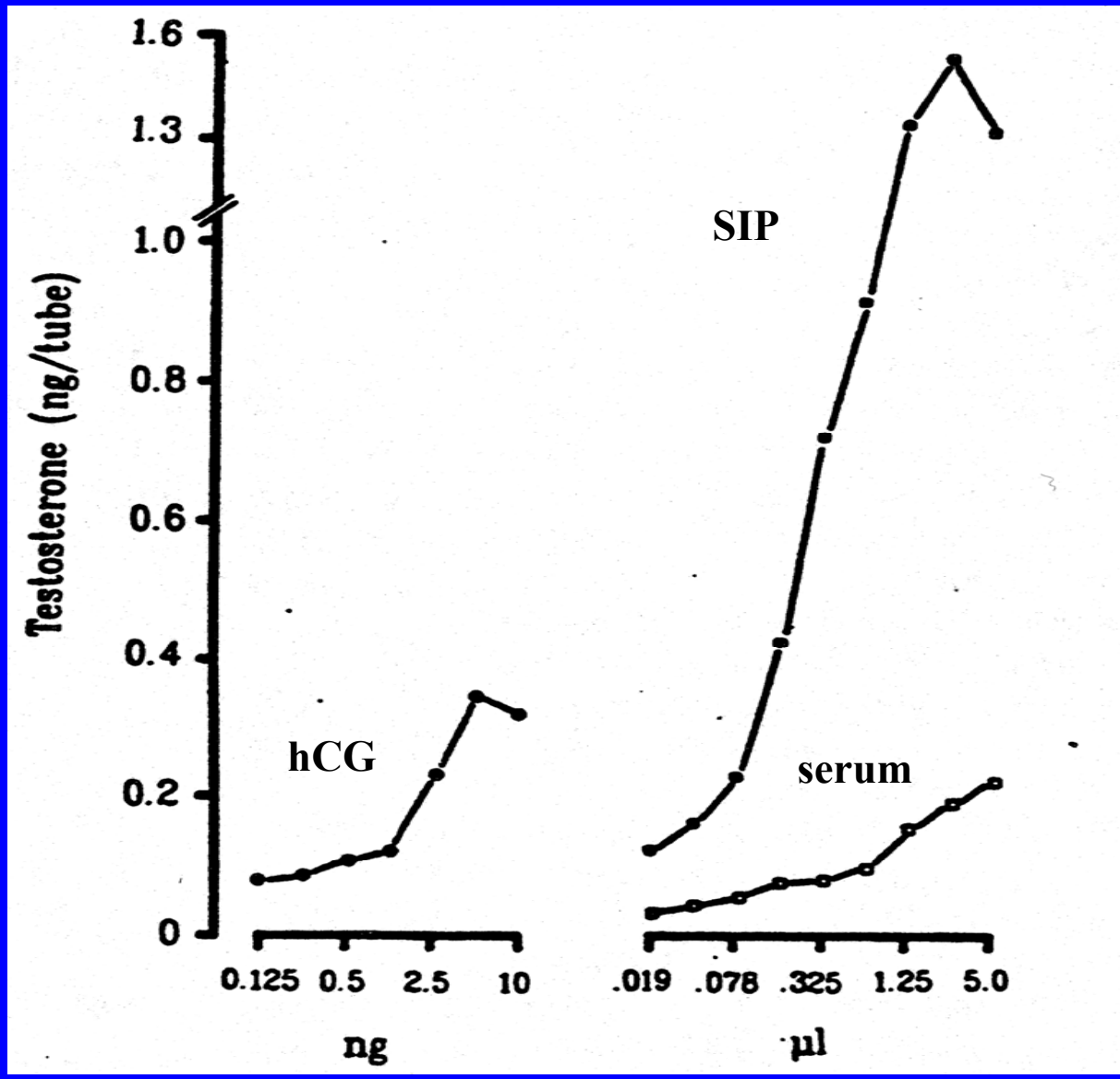
Leydig Cells (60-day old rats)

↓

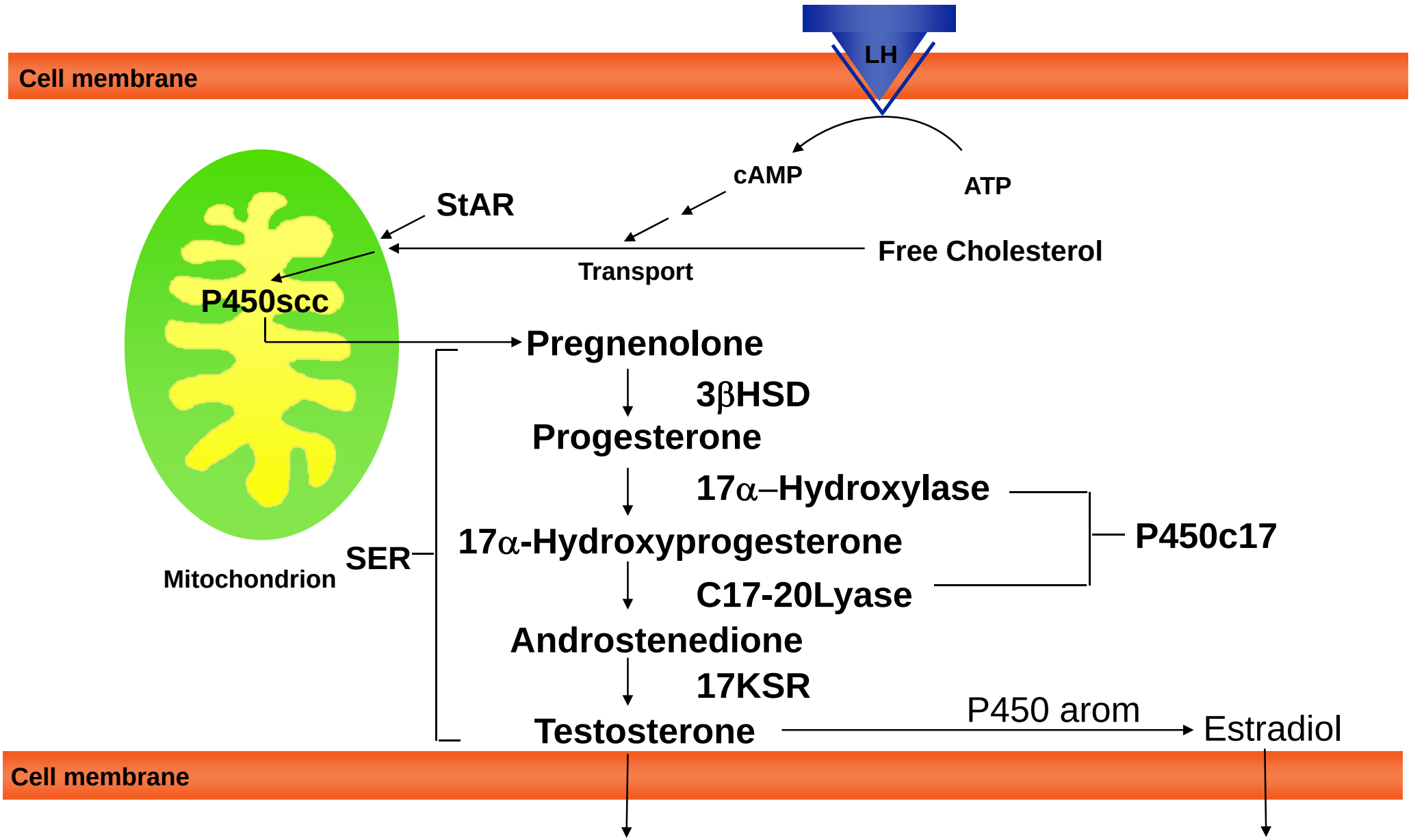
Incubated in the presence of hCG, hFF and serum proteins for 3 hours

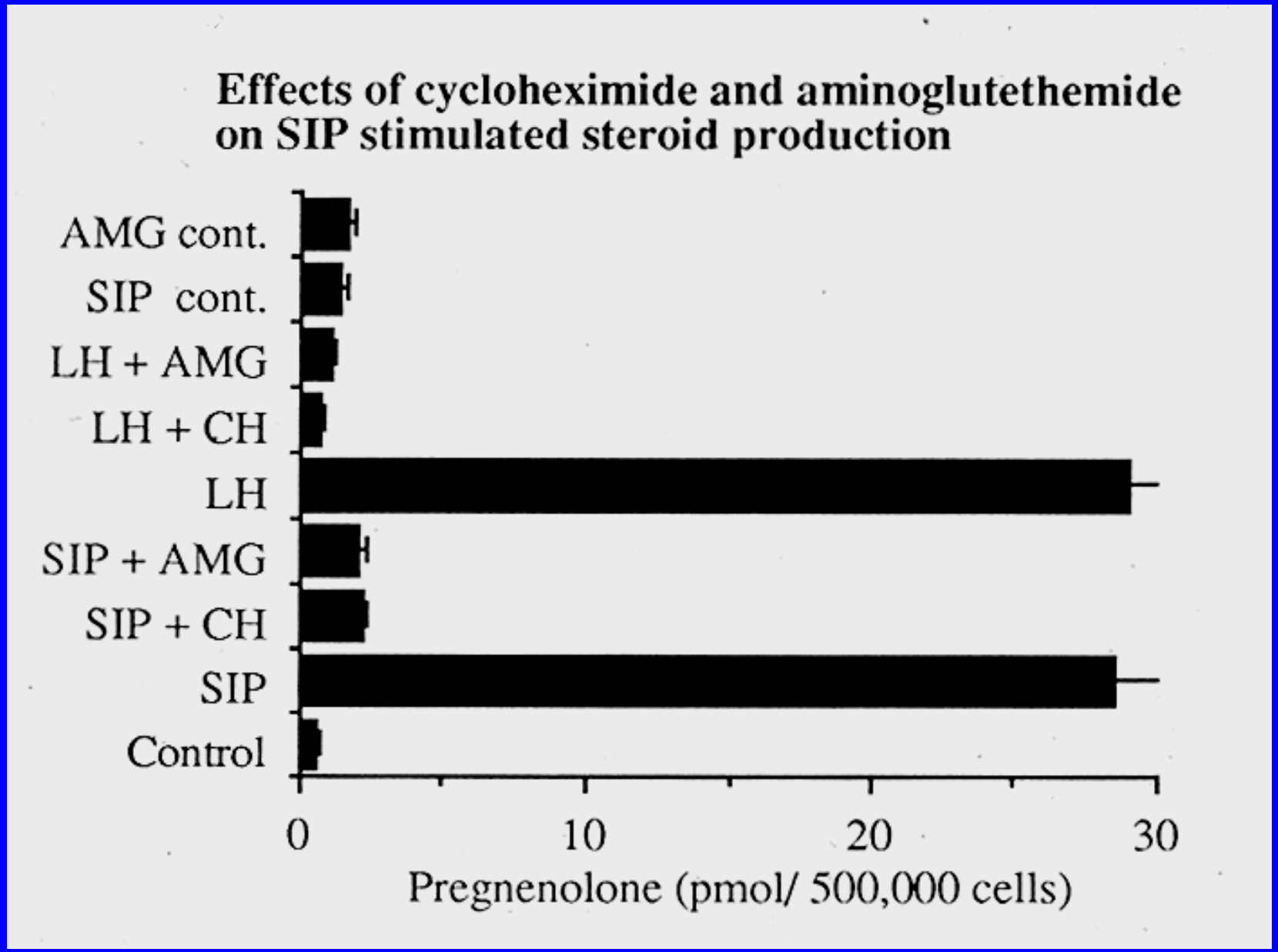
↓

Determination of testosterone by RIA

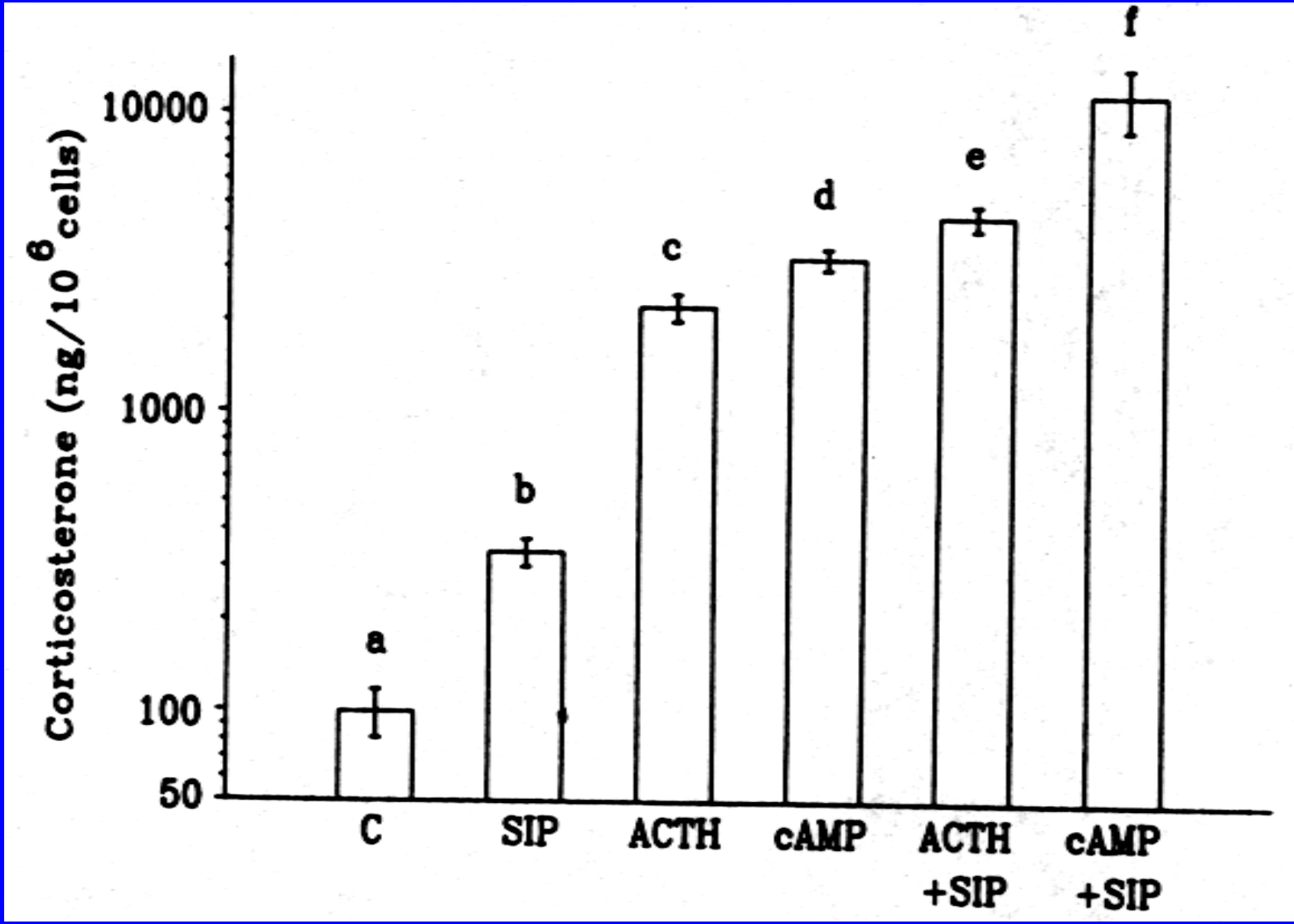


LH stimulated testosterone production in Leydig cells



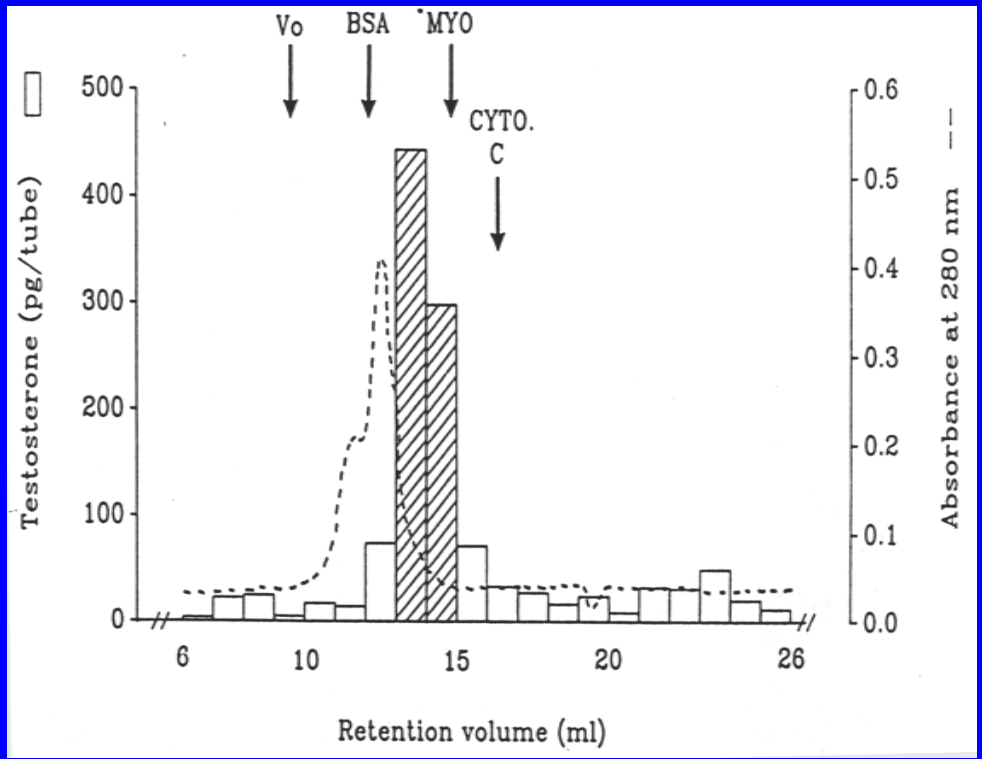

Effects of cycloheximide and aminoglutethemide on SIP stimulated steroid production
AMG cont.
SIP cont.
LH + AMG
LH + CH
LH
SIP + AMG
SIP + CH
SIP
Control
0
10
20
30
Pregnenolone (pmol/ 500,000 cells)

Effects of hFF proteins on Steroidogenesis in Rat Adrenal cells

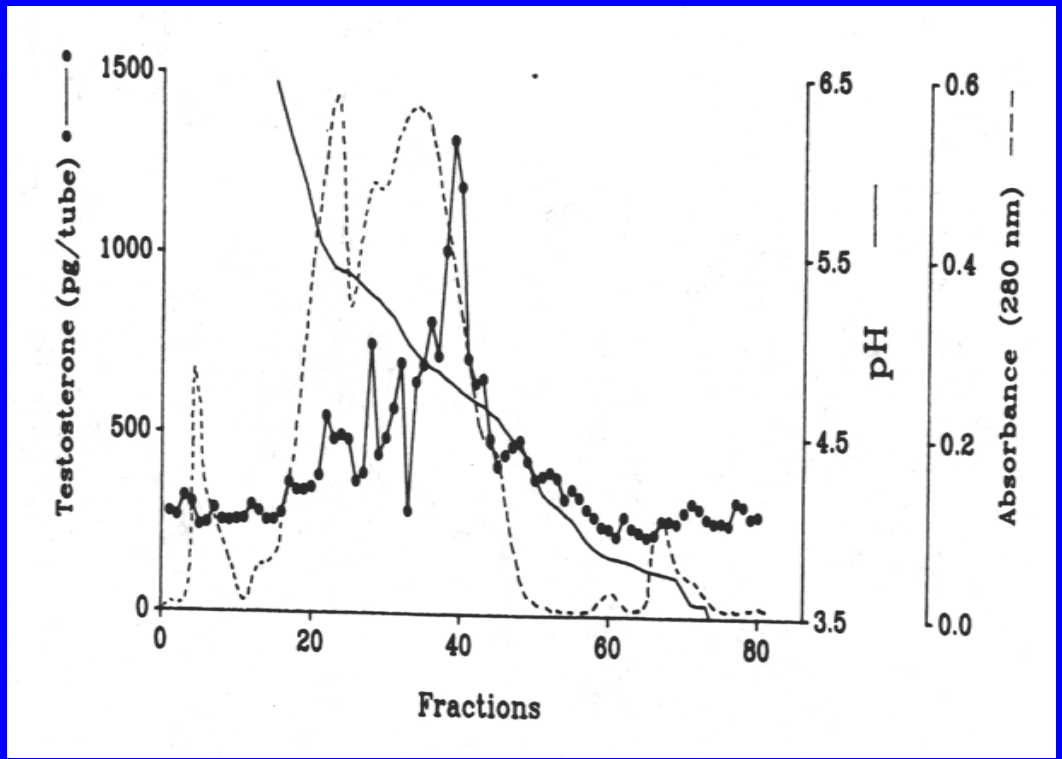


Physico-chemcial Properties of Steroidogenic Factors in hFF

Gel Chromatography

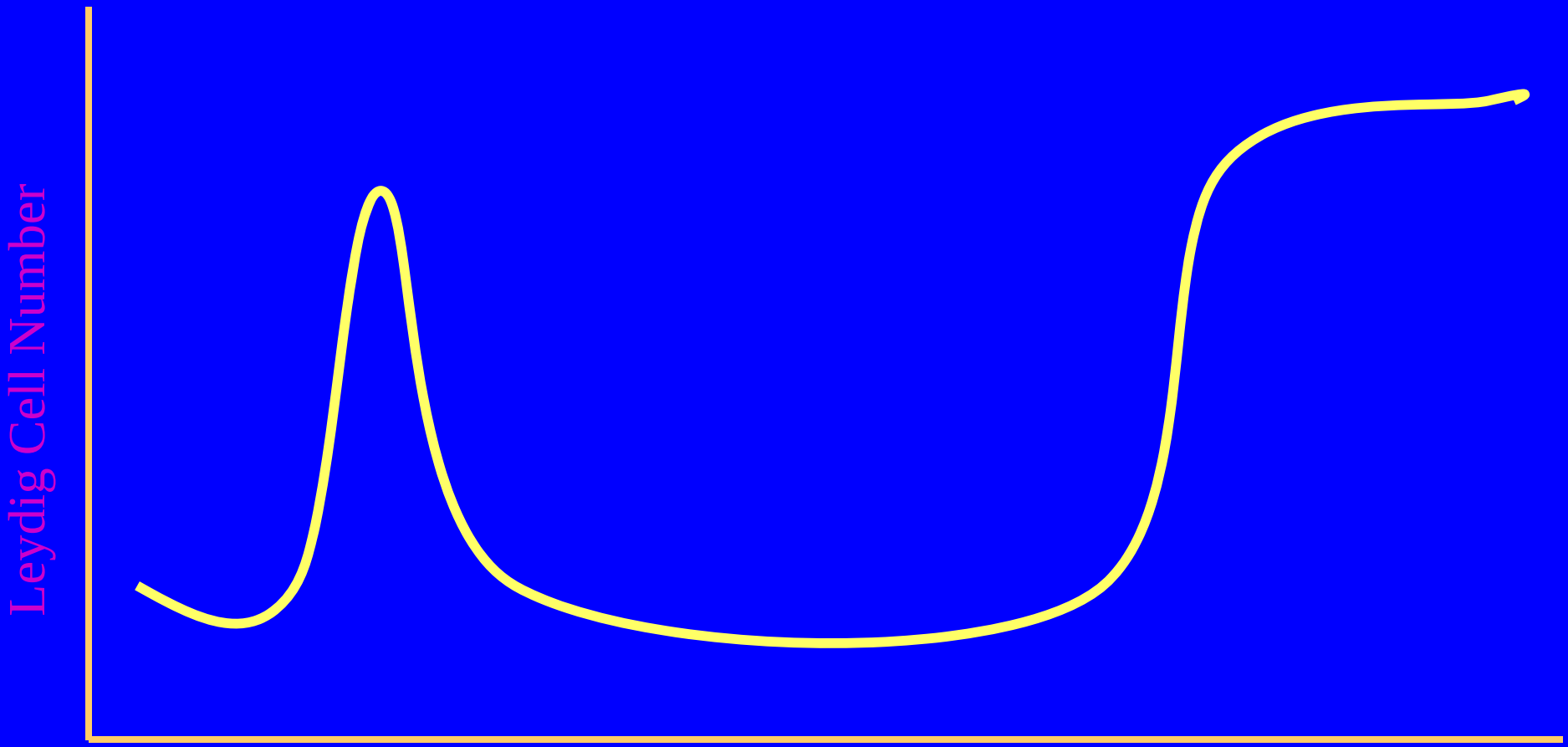


Isoelectrofocusing

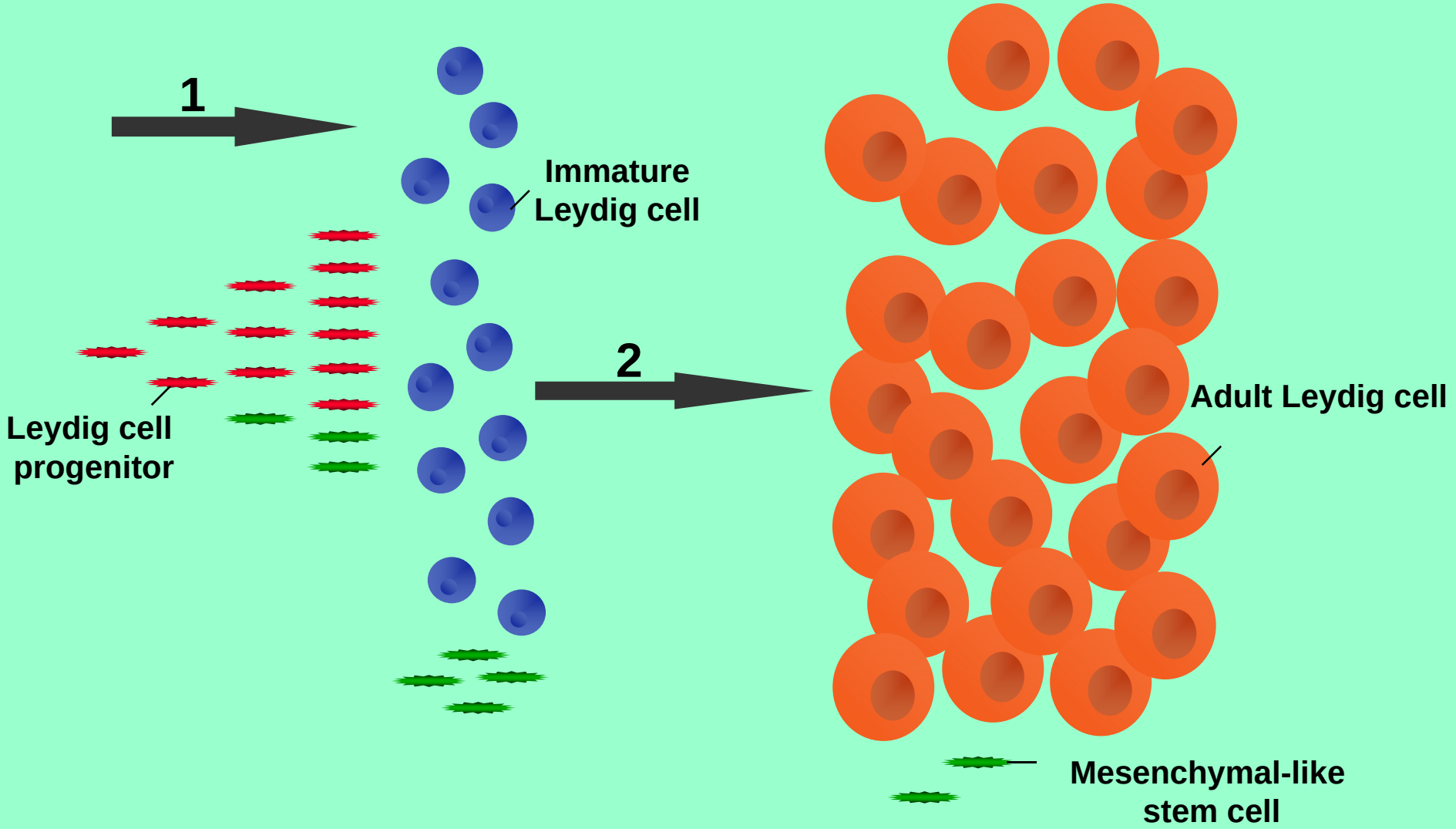


- Steroidogenesis - Inducing Protein (SIP)

Life History of Leydig Cells



Stages of Leydig Cell Development



SIP Stimulates DNA Synthesis in Rat Leydig Cells

Immature rat Leydig Cells

⇩

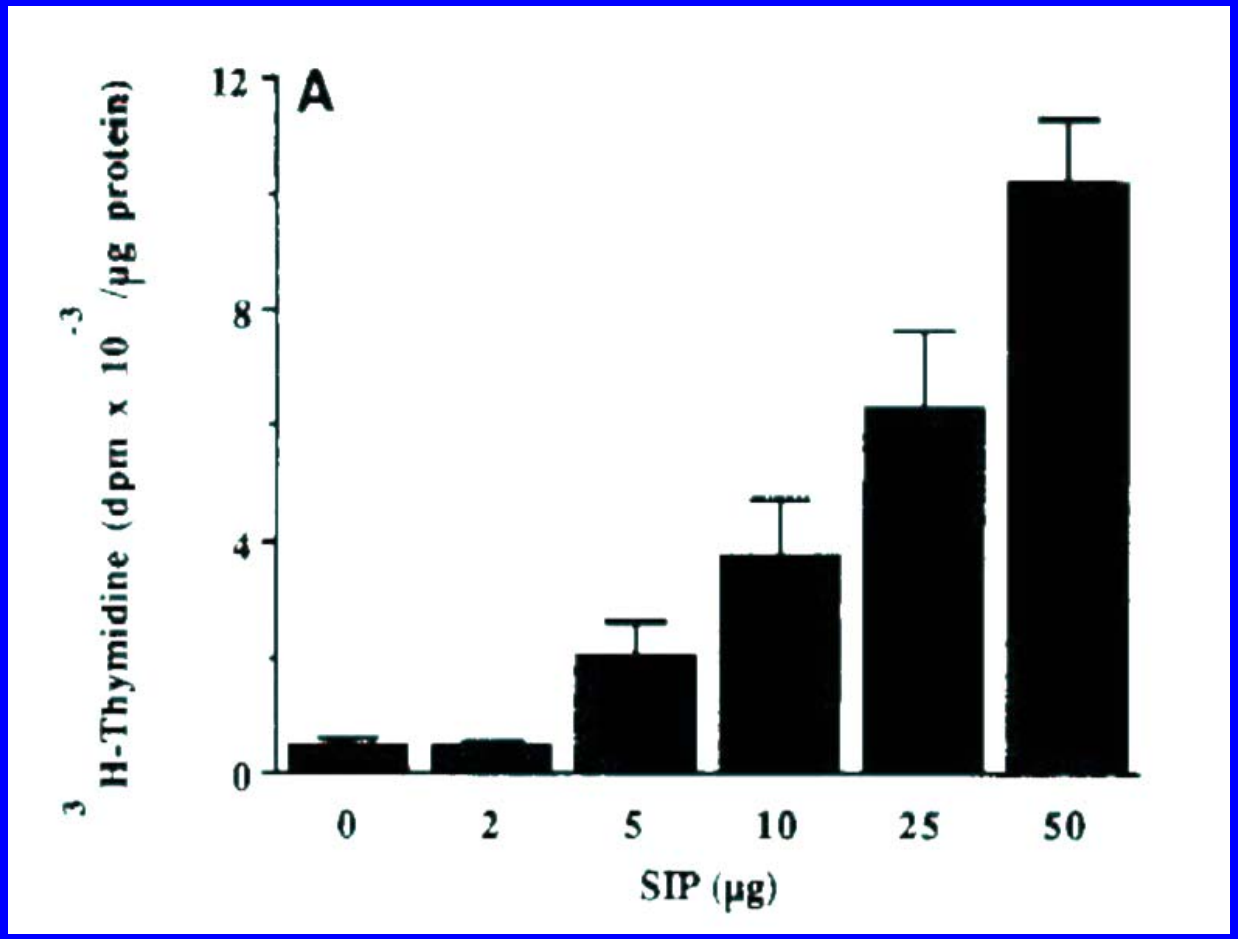
Cultured with different doses of SIP for 18 h

⇩

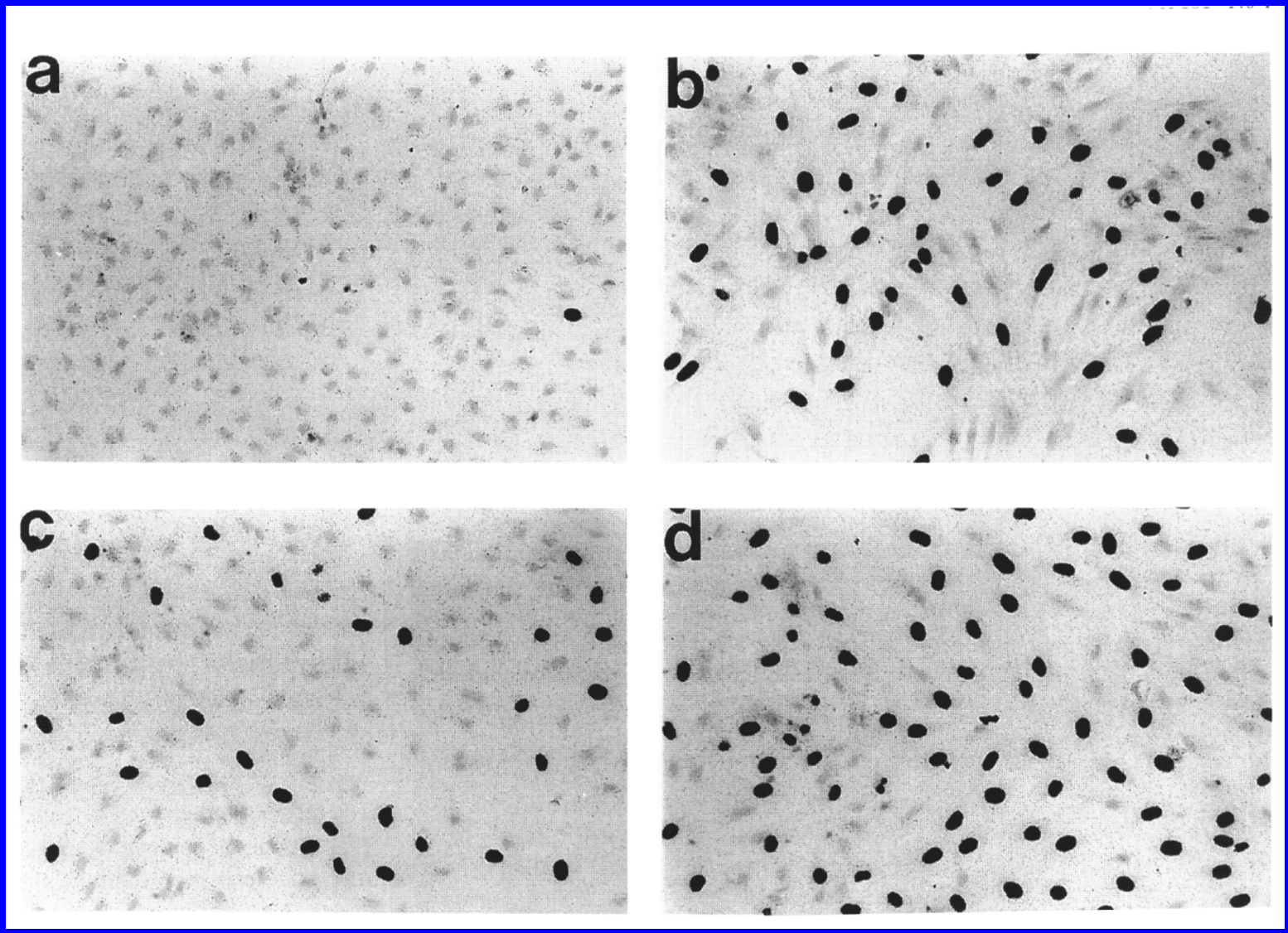
Washed and incubated with ^{3}H-thymidine for 4 h

⇩

Determined incorporation of ^{3}H thymidine into DNA



Autoradiography of labeled Leydig Cells



C

IL-1

SIP

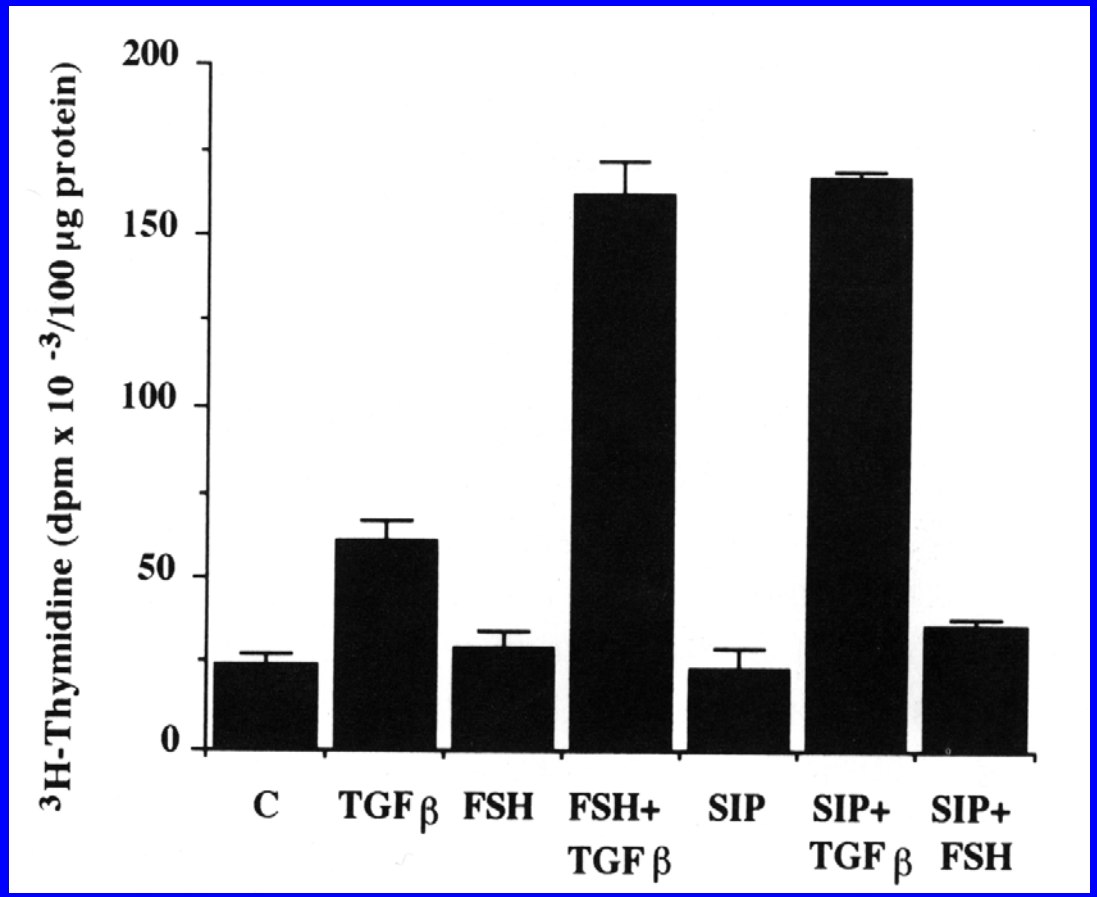
IL-1 + SIP

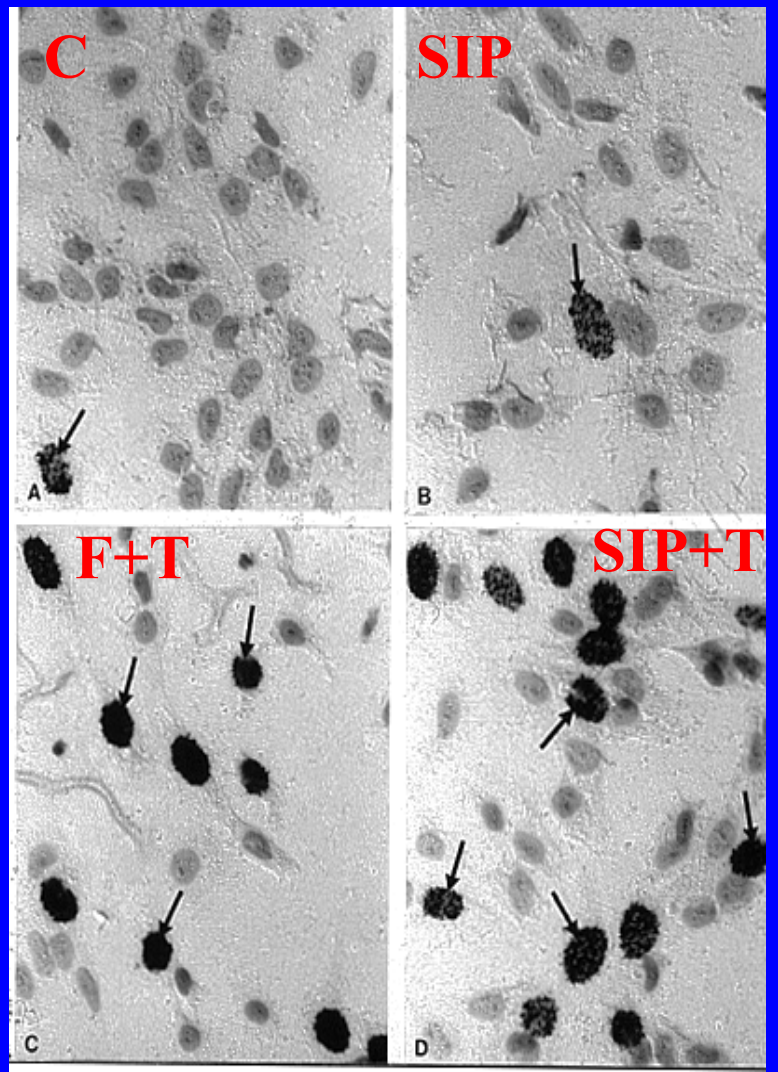
Effects of SIP on Ovarian Cells

1. Effects of SIP on proliferation of rat granulosa cells.

2. Effects of SIP on proliferation of human ovarian epithelial cancer cells.

SIP Interacts with TGFβ to Stimulate DNA Synthesis in Rat Granulosa Cells





Ovarian Epithelial Cancer

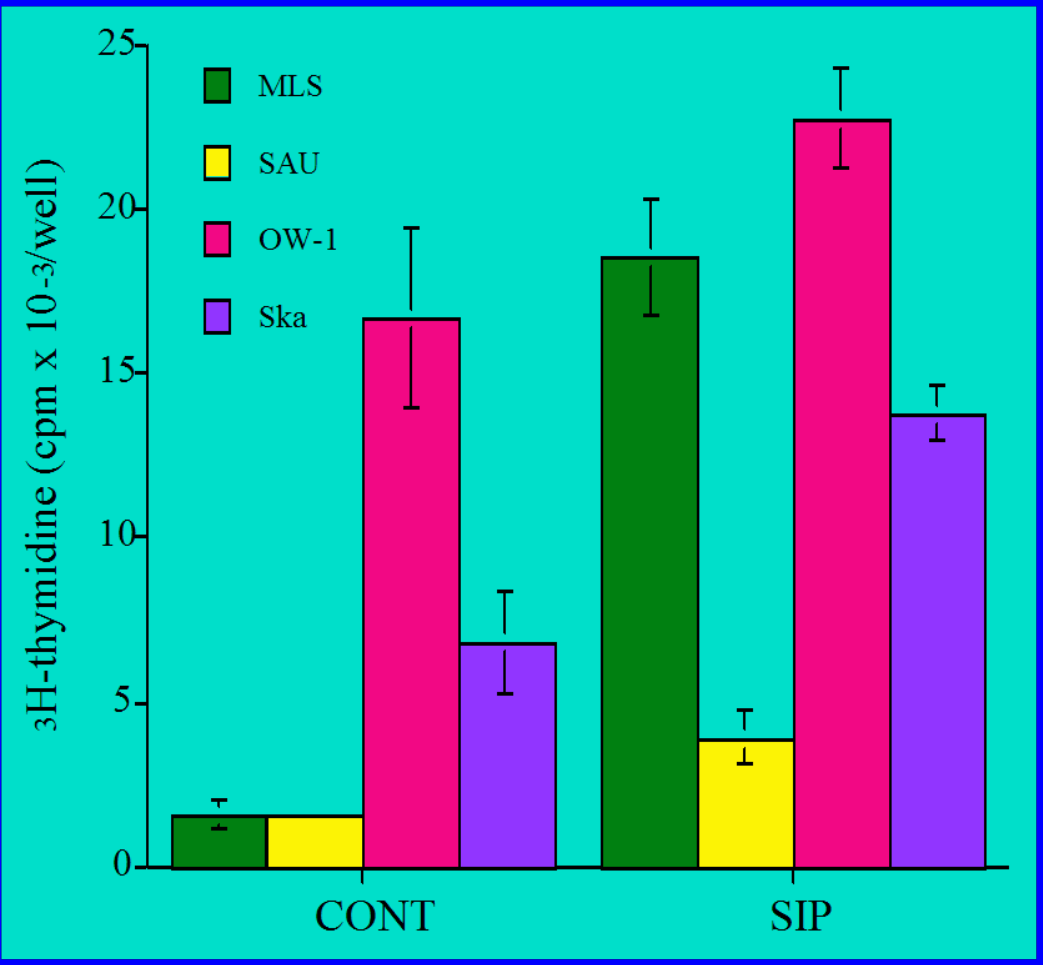
- **INCREASED RISK ASSOCIATED WITH**
  1. NUMBER OF OVULATIONS
  2. USE OF FERTILITY DRUGS

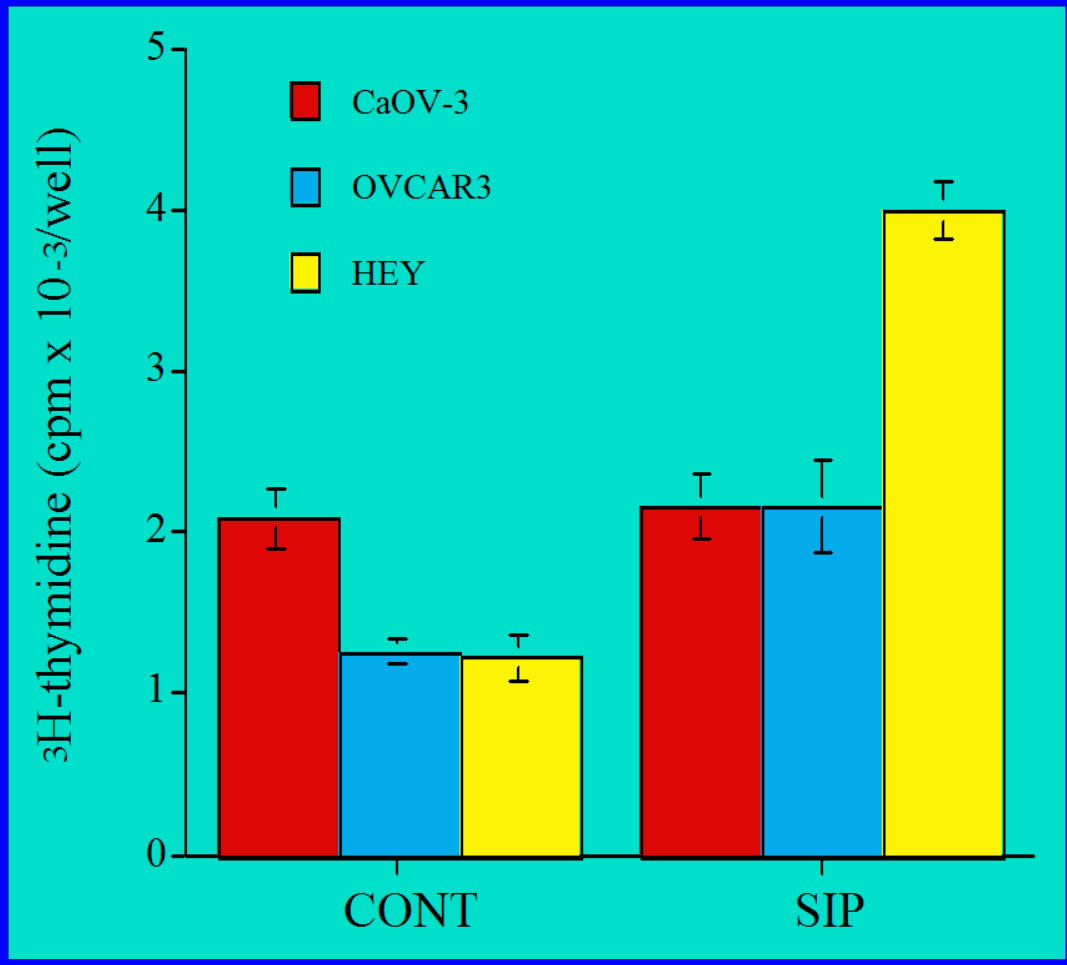
- **REDUCED RISK ASSOCIATED WITH**
  1. MULTIPLE PREGNANCIES AND LACTATION
  2. ORAL CONTRACEPTIVES

Ovarian Epithelial Cancer Cell Lines

1. HEY CELLS
2. OVCAR- 3 CELLS
3. CAOV-3 CELLS
4. SKA CELLS
5. MLs
6. OW-1
7. Sau

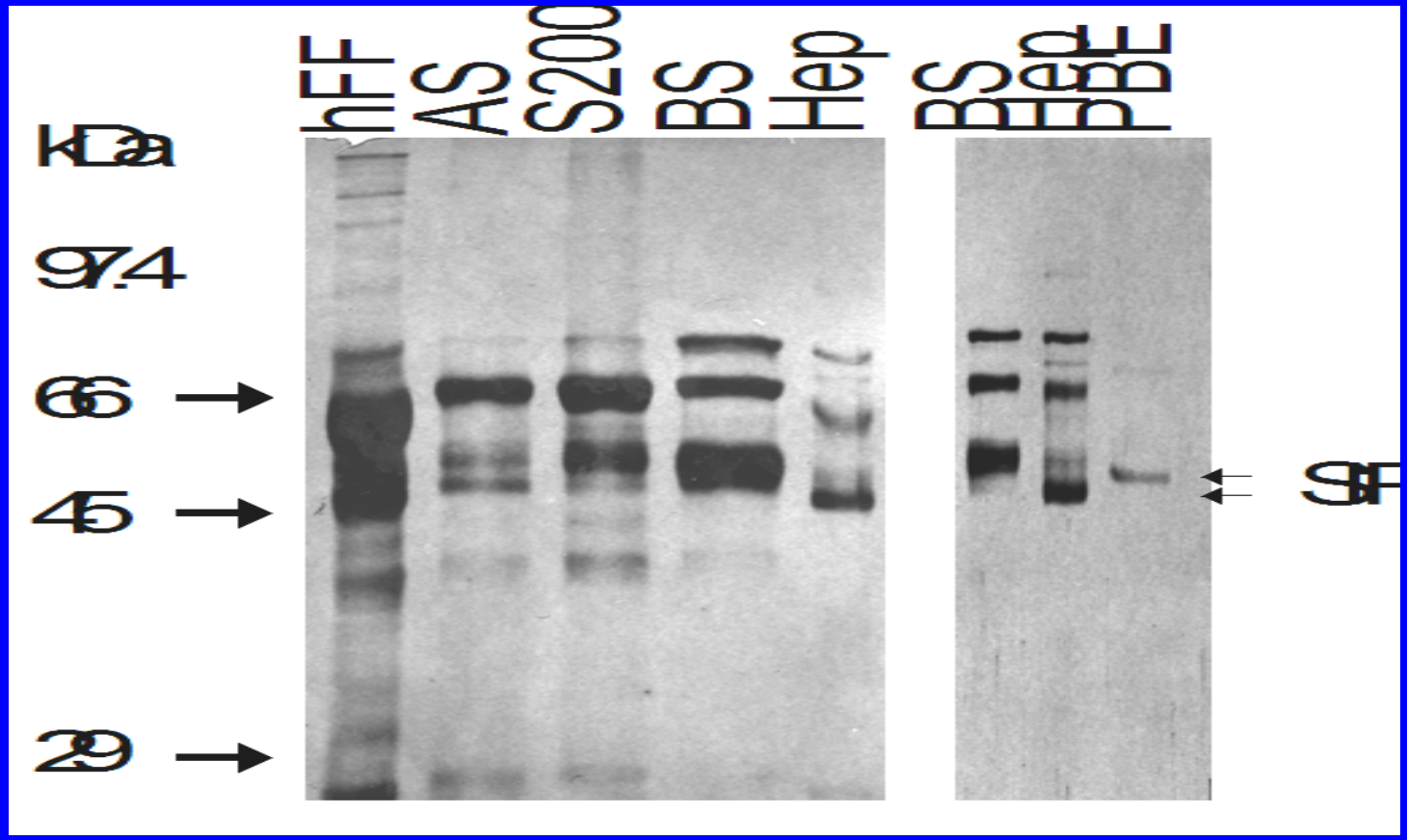
Effects of SIP on DNA Synthesis in Ovarian Surface Epithelial Cell Lines





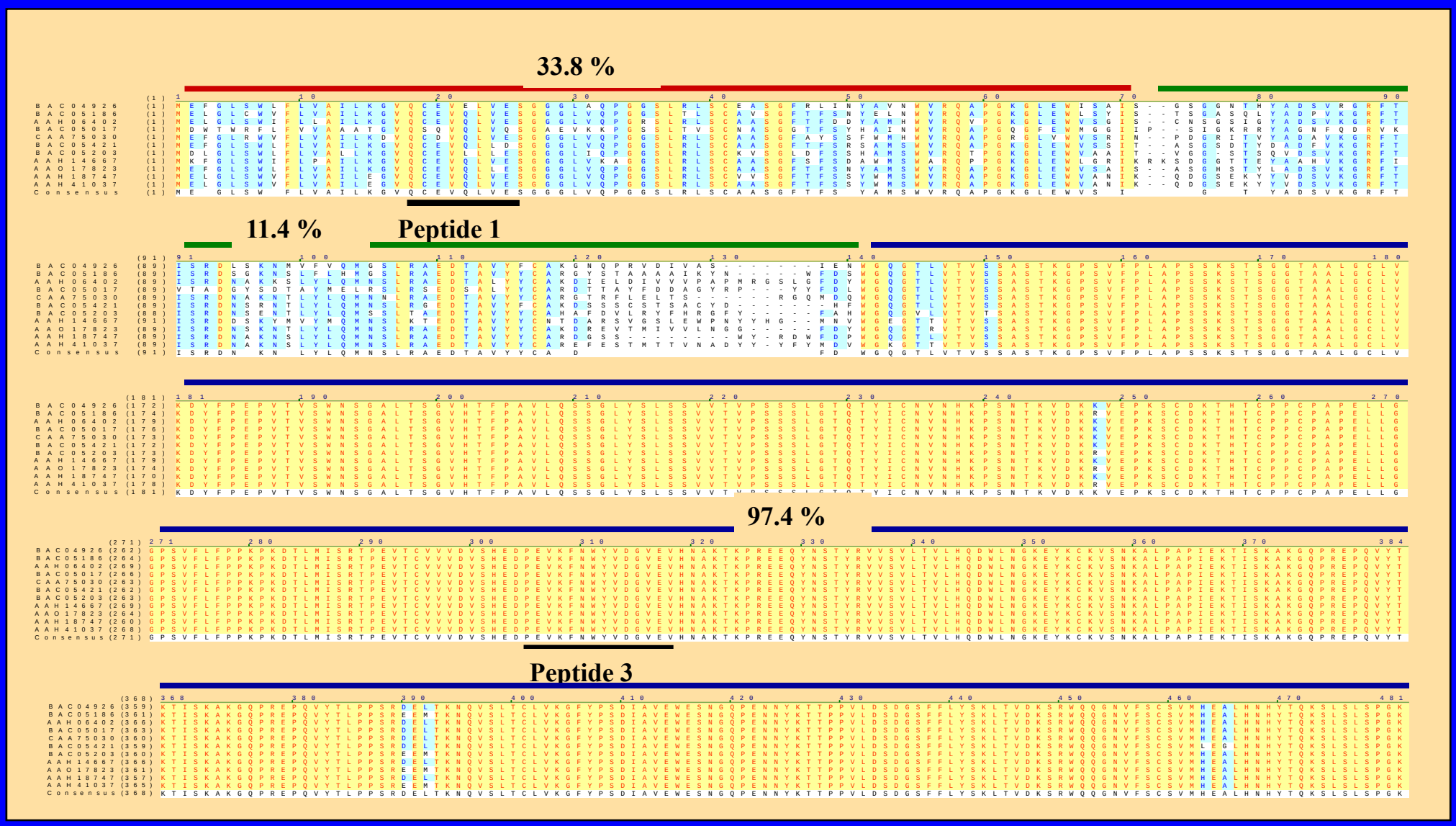
Biochemical and Molecular Characterization of SIP

Purification and Amino Acid Sequence of SIP



EVQLVESG	**Peptide 1**
DVNGGGATLPQPLYQTA	**Peptide 2**
FNWYVDGVEVHNAK	**Peptide 3**

Conserved SIP Peptide Sequences Found in Protein Databases



Homology of SIP with other Human cDNAS

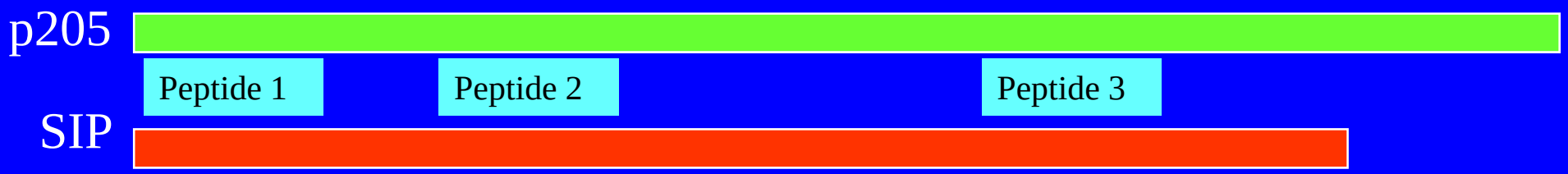
Accession number	Tissue/Cell Source	Function	Molecular Weight (kDa)
BAC04926	small intestine	unknown	51.3
BAC05186	thymus	unknown	51.6
AAH06402	primary B-Cells from tonsils	similar to IgG heavy constant gamma 3 (G3m marker)	52.2
BAC05017	synovial membrane tissue from rheumatioid	unknown	52.2
CAA75030	human /mouse (NS-0) hetero-hybridoma cell line	IgG kappa heavy chain	52.0
BAC05421	synovial membrane (knee)	unknown	51.3
BAC05203	thymus	unknown	51.7
AAH14667	spleen	similar to IgG heavy constant gamma 3 (G3m marker)	52.5
AAO17823	serum	anti-rabies SOJA IgG heavy chain	51.7
AAH18747	primary B-Cells from tonsils	similar to IgG heavy constant gamma 3 (G3m marker)	51.7
AAH41037	primary B-Cells from tonsils	similar to IgG heavy chain 4 (serum IgG1)	52.6

DING Proteins

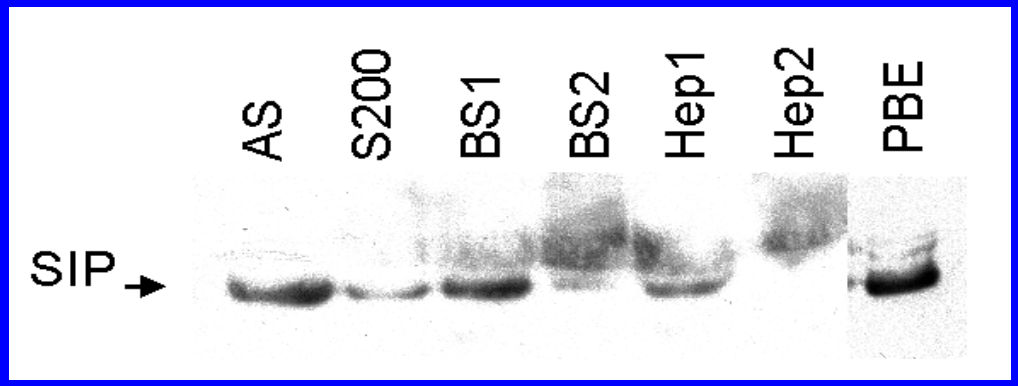
Protein	Tissue Source	Peptide Sequence
SIP, human **S**teroidogenesis-**I**nducing **P**rotein, 50KD	Human Follicular Fluid; Human Granulosa-Luteal cells; Human Ovarian Epithelial Cancer Cells	**DVNGGG**ATLPQPLYQTA
p205, human synovial stimulatory protein	Rheumatoid Arthritis Synovial Fluid	**DINGGG**ATLPQPLYQTAAVLTAGFA
HSFP, 40KD protein	Human fibroblast cells, cervical carcinoma cells	**DINGGG**ATLPQPLYQTSGVLTAGFAPYTGF
38KD protein	Human Breast Cancer Cell Line MCF7	**DINXGG**ATLPQPLYQTXGVLPAGFAPYIGVSXXG
CAI, Crystal **A**dhesion **I**nhibitor, 39KD	Renal epithelial cells	**DINGGG**ATLPQPLYQTSGVLTAGFAPYISVNAK
p40, cotinine receptor	Rat neurons	**XINGGG**ATLPQKLYLTPNVLTAGFAPYI

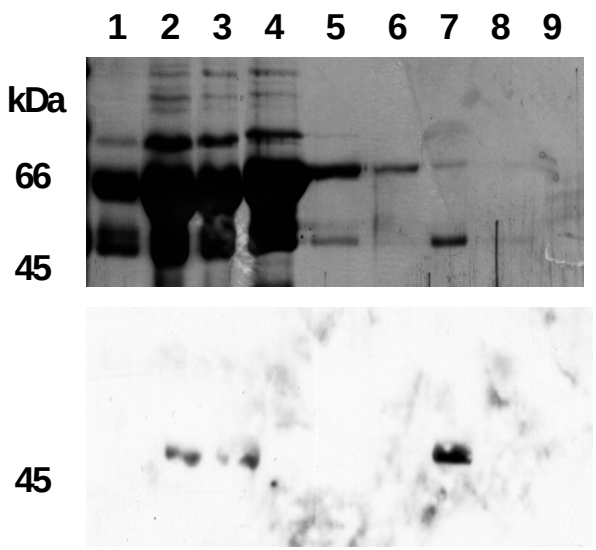
Comparison of SIP and P205 Sequences

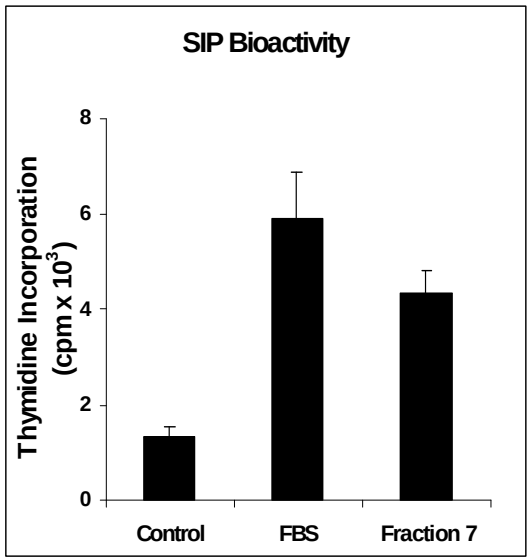
	Peptide 1	Peptide 2	Peptide 3
p205	XVQLVE	DINGGGATLPQPLYQTAGVLTAGFA	YVDGVEVHNAK
SIP	EVQLVESG	DVNGGGATLPQPLYQTA	FNWYVDGVEVHNAK



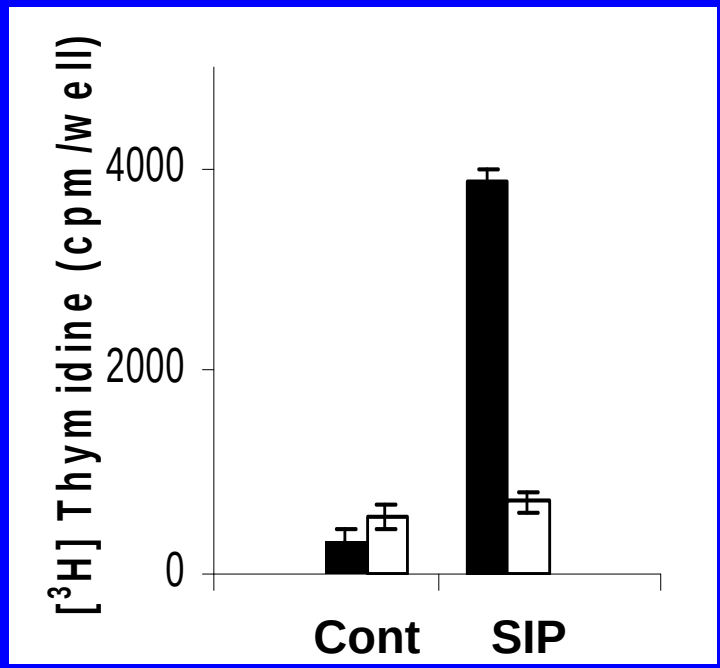
Anti-p205 Cross-react With Bioactive SIP



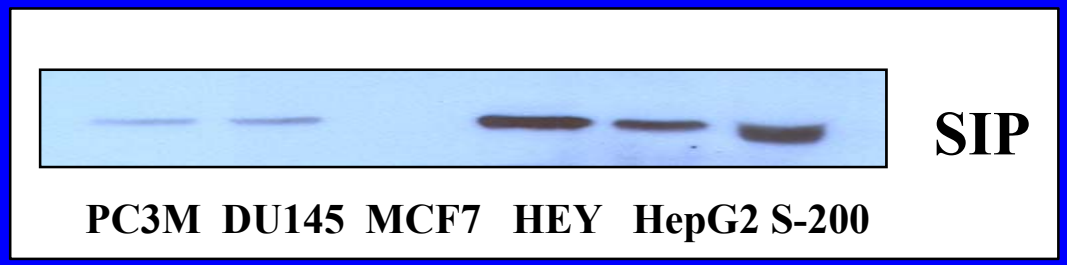


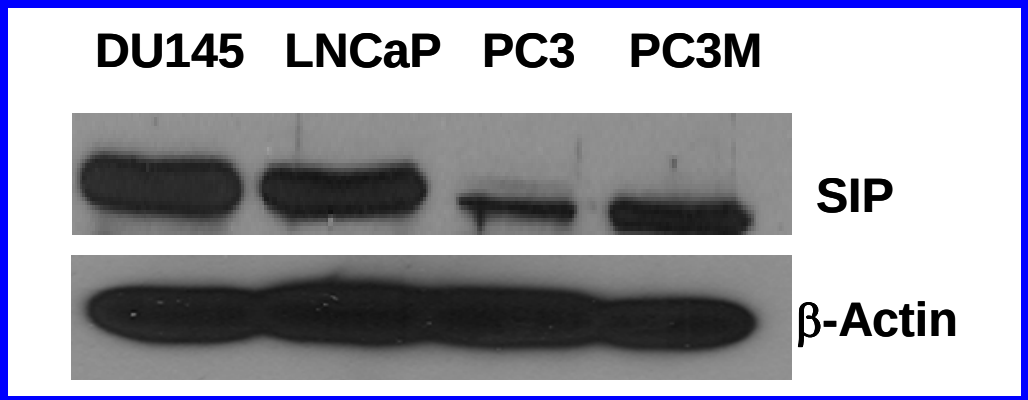


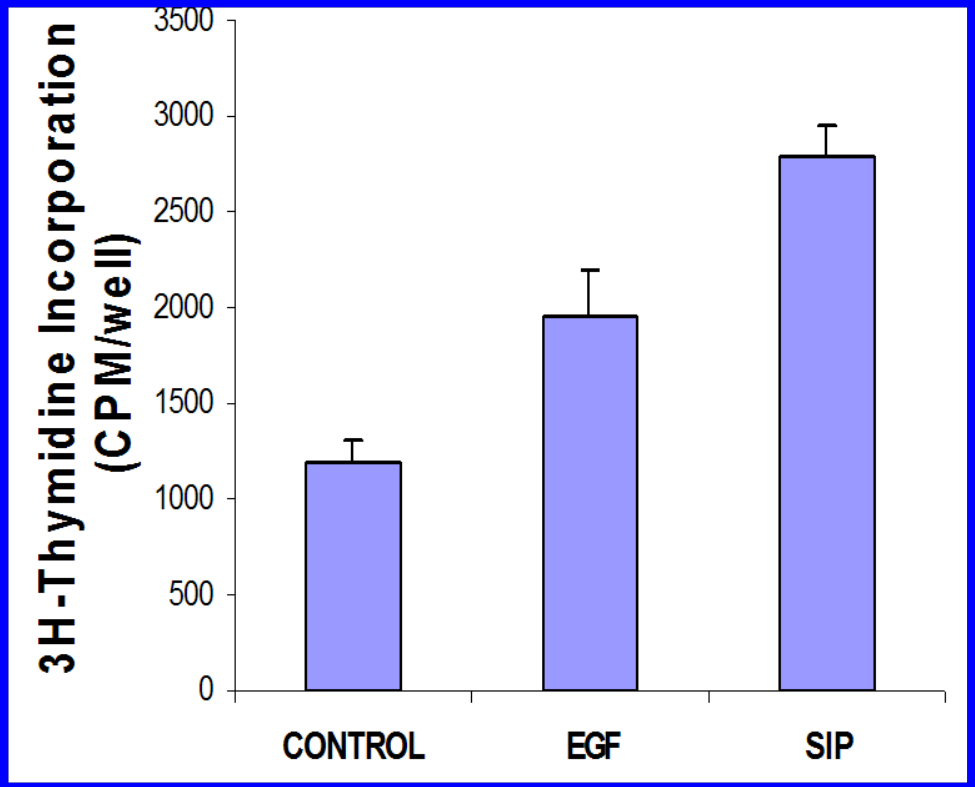
EVQLVESG



SIP production and effects on cancer cells

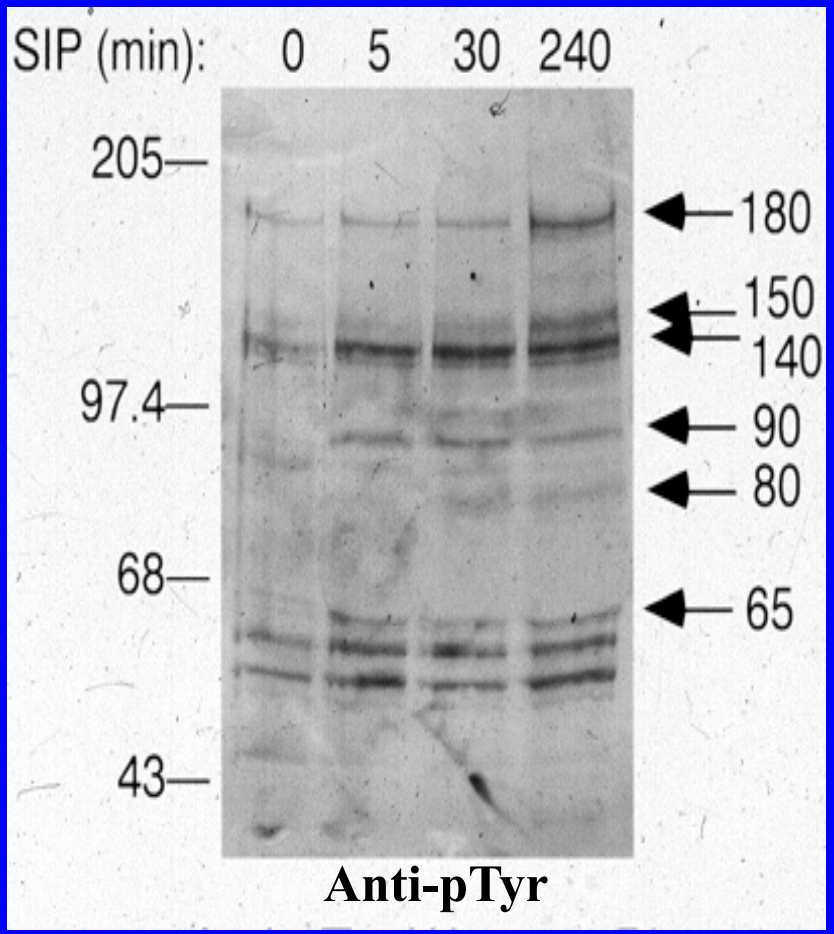




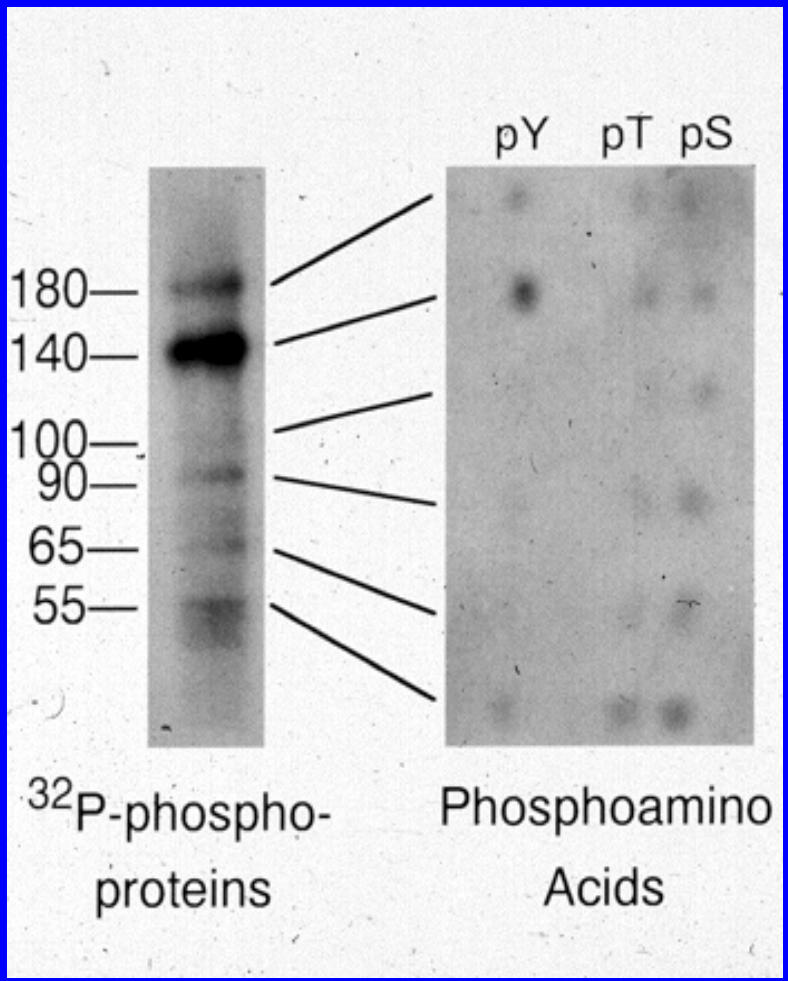


Mechanism of Action of SIP

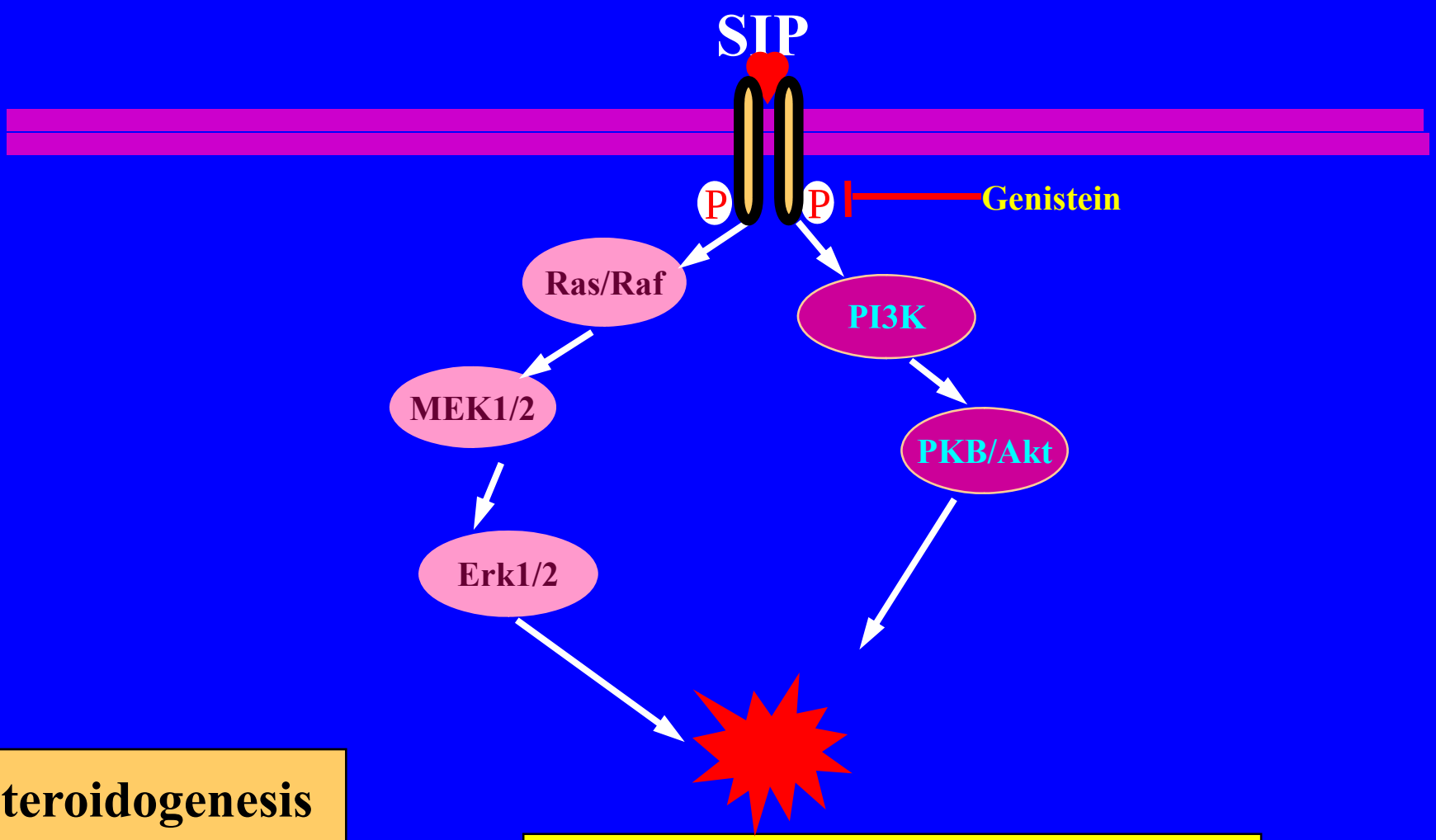
SIP-induced Tyrosine Phosphorylation in immature rat Leydig cells



Anti-pTyr

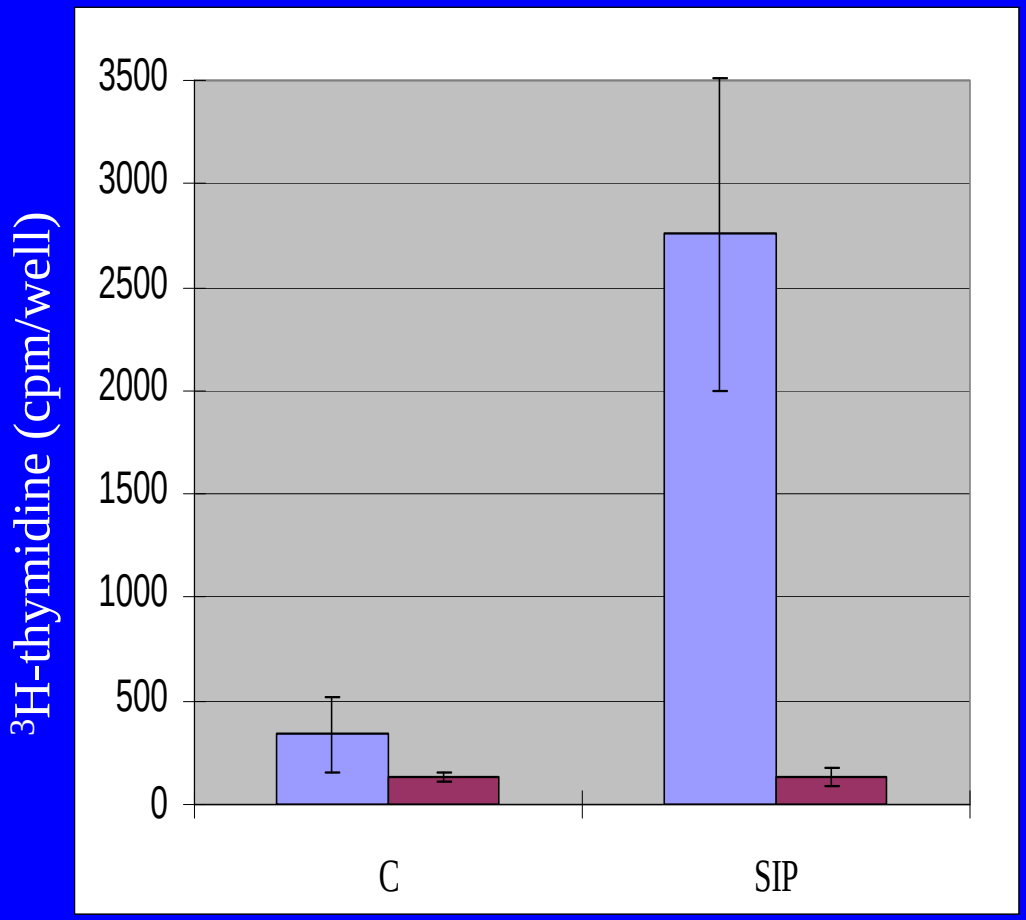


Mechanism of SIP Action

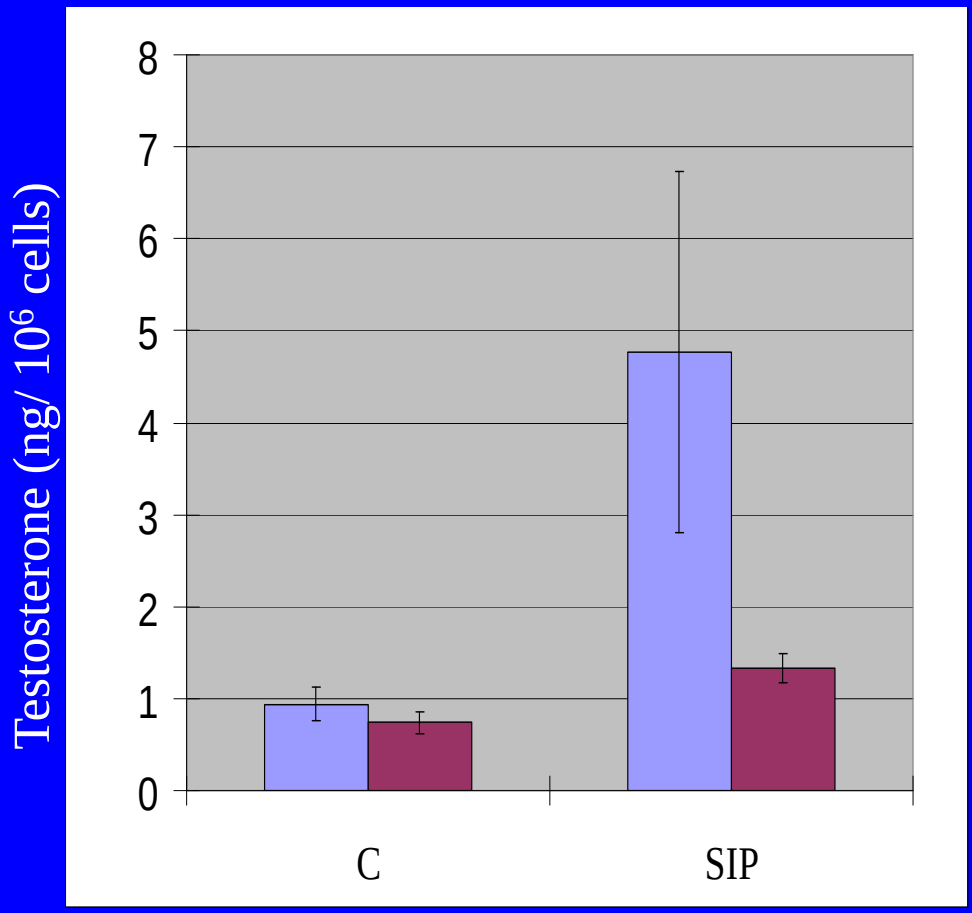


Genistein Blocks the Effects of SIP on DNA Synthesis and Steroidogenesis in Leydig Cells

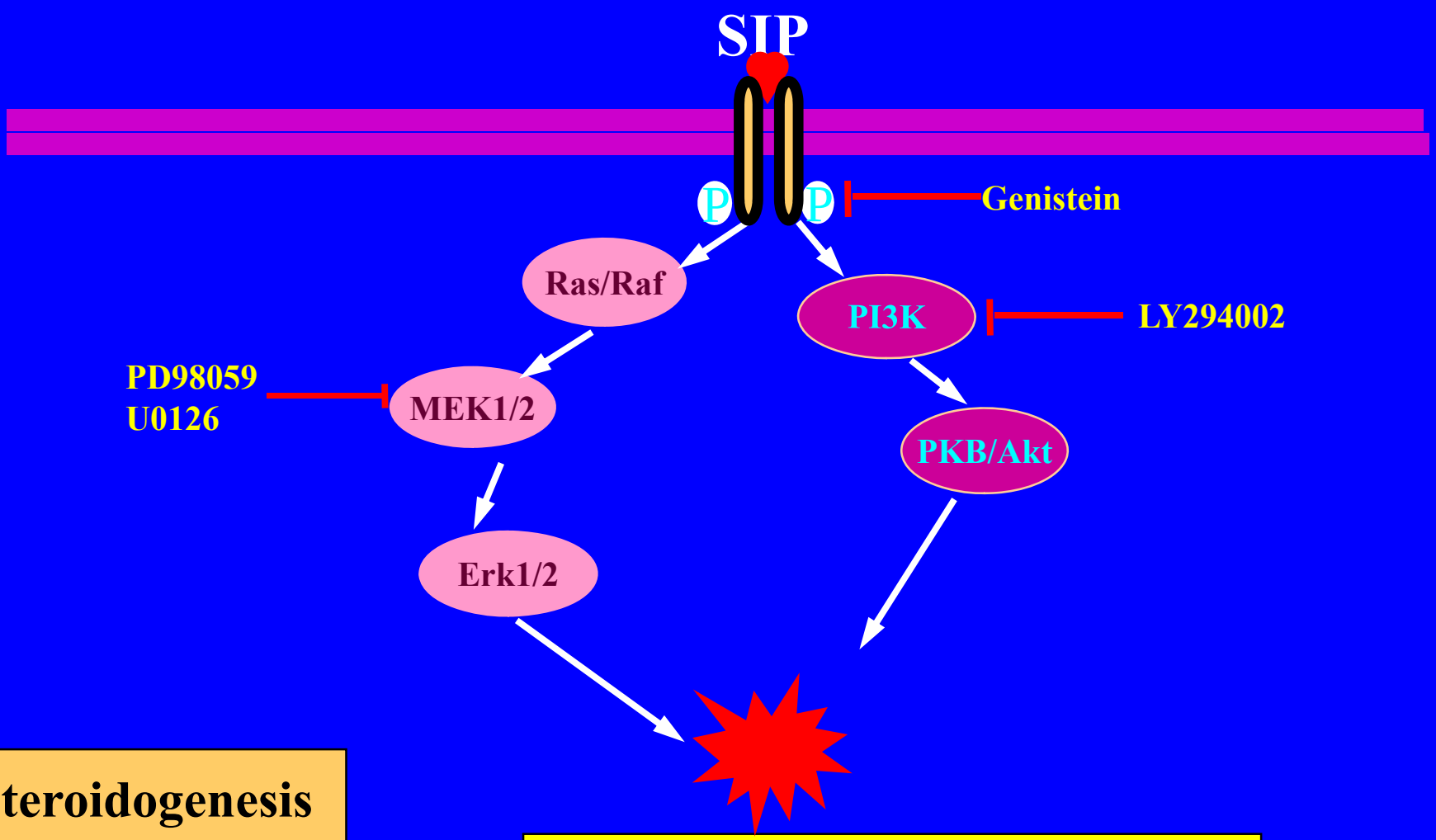
DNA Synthesis



Steroidogenesis

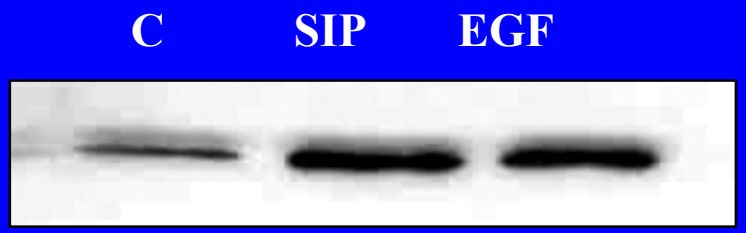


Mechanism of SIP Action

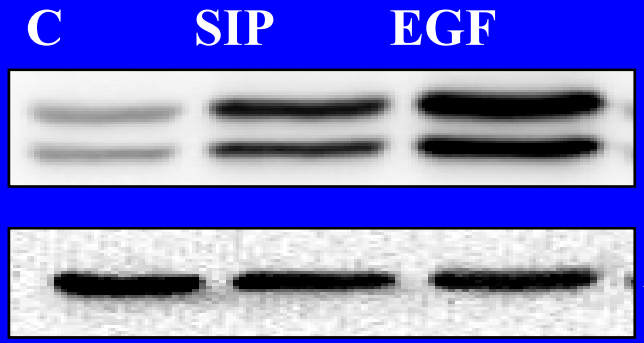


Ras-Raf-MAP Kinase signaling pathway

SIP effects on the activation of MAP-Kinase in ILC cells

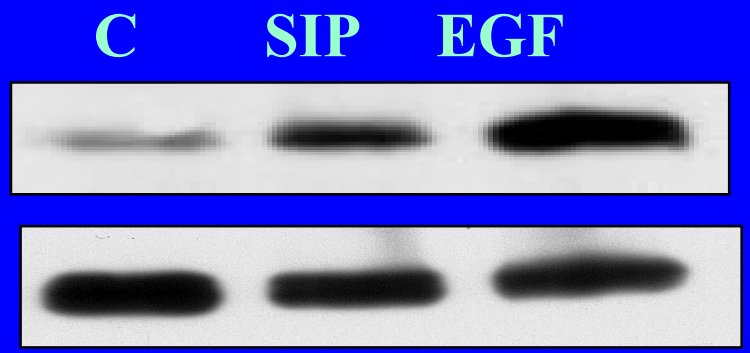


Ras-GTP



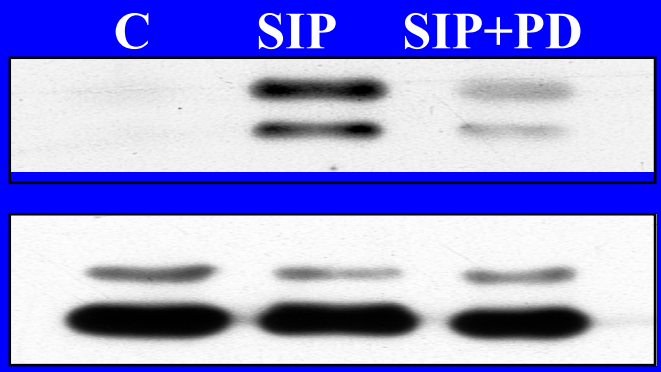
p-ERK1
p-ERK2

Actin



p-MEK

MEK

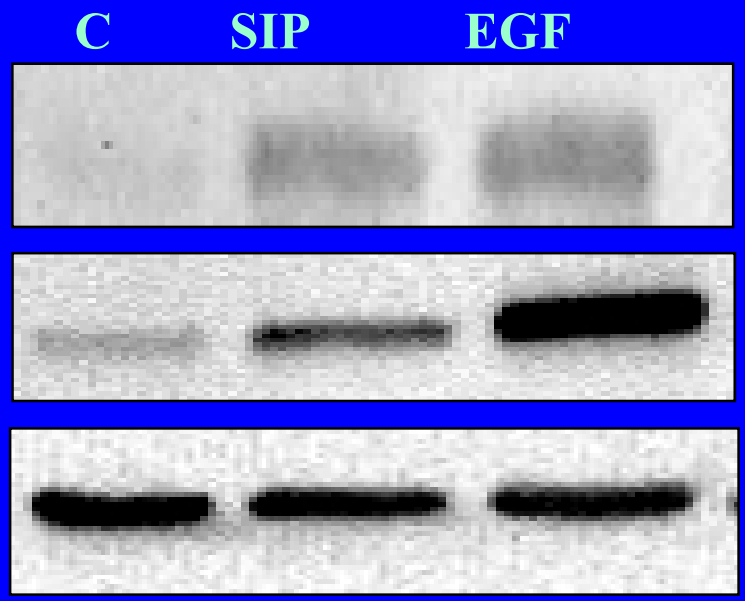


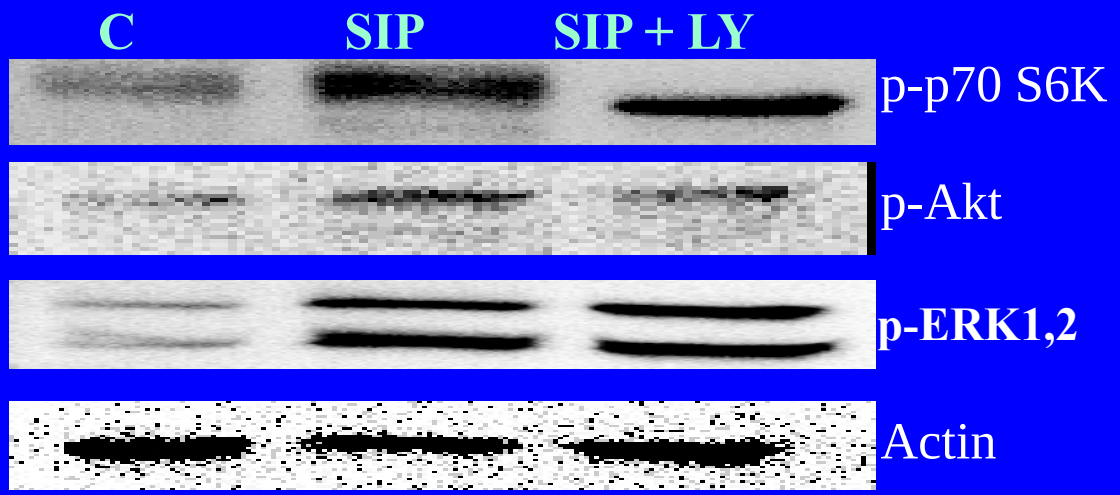
p-ERK1
p-ERK2

Pan-ERK

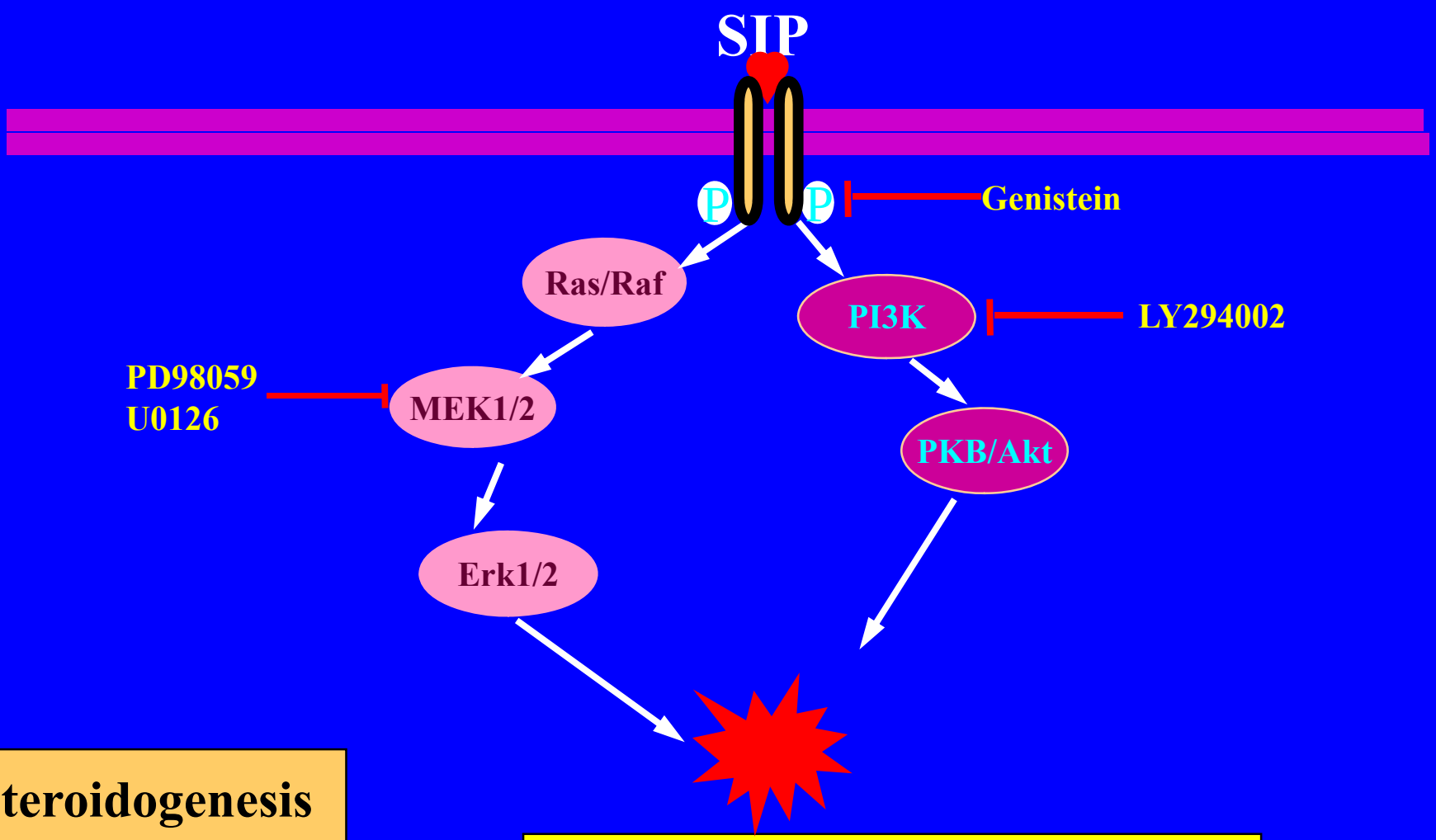
PI3 Kinase-PKB/Akt Signaling Pathway

SIP effects on the activation of Akt in ILC cells



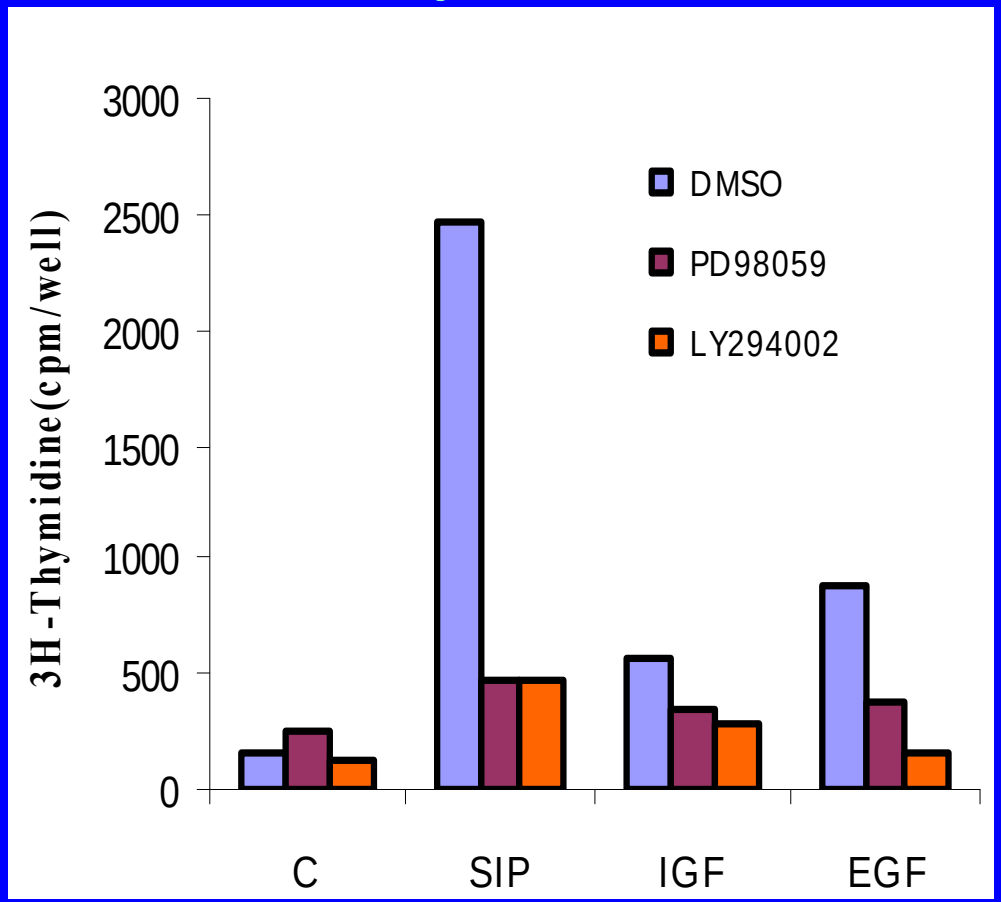


Mechanism of SIP Action

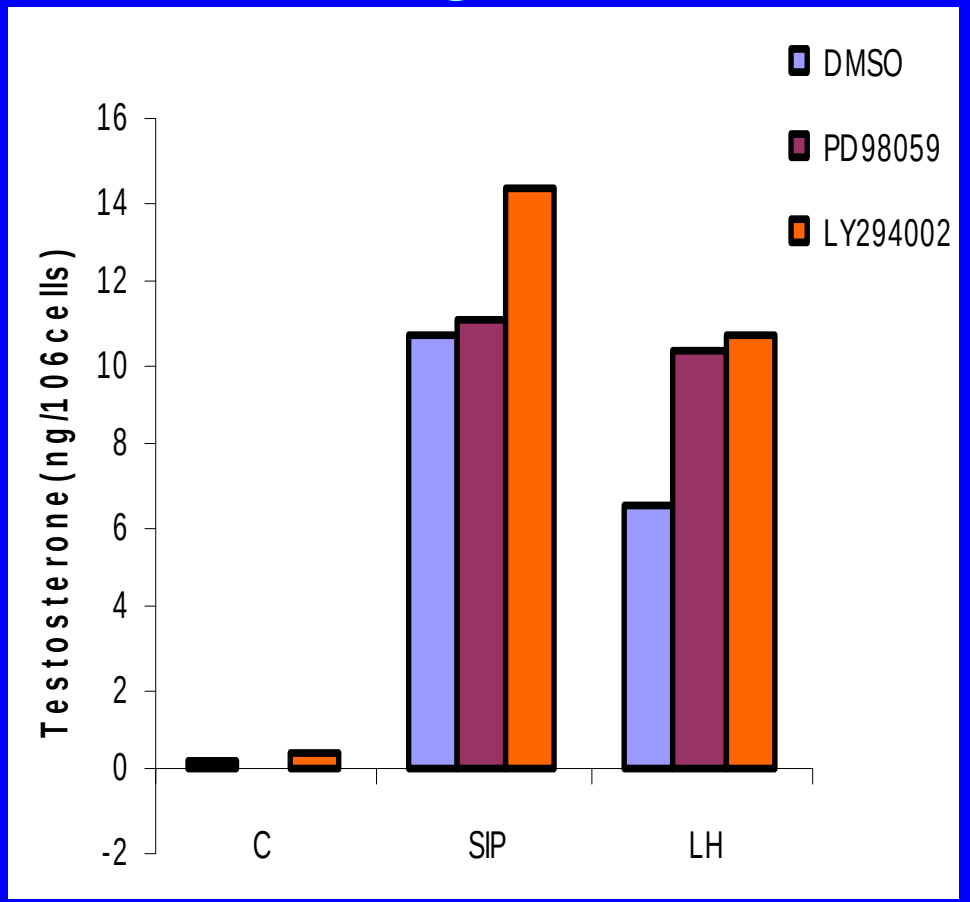


Effects of SIP on DNA Synthesis and Steroidogenesis in Leydig Cells in the Presence of MEK and PI3-Kinase Inhibitors

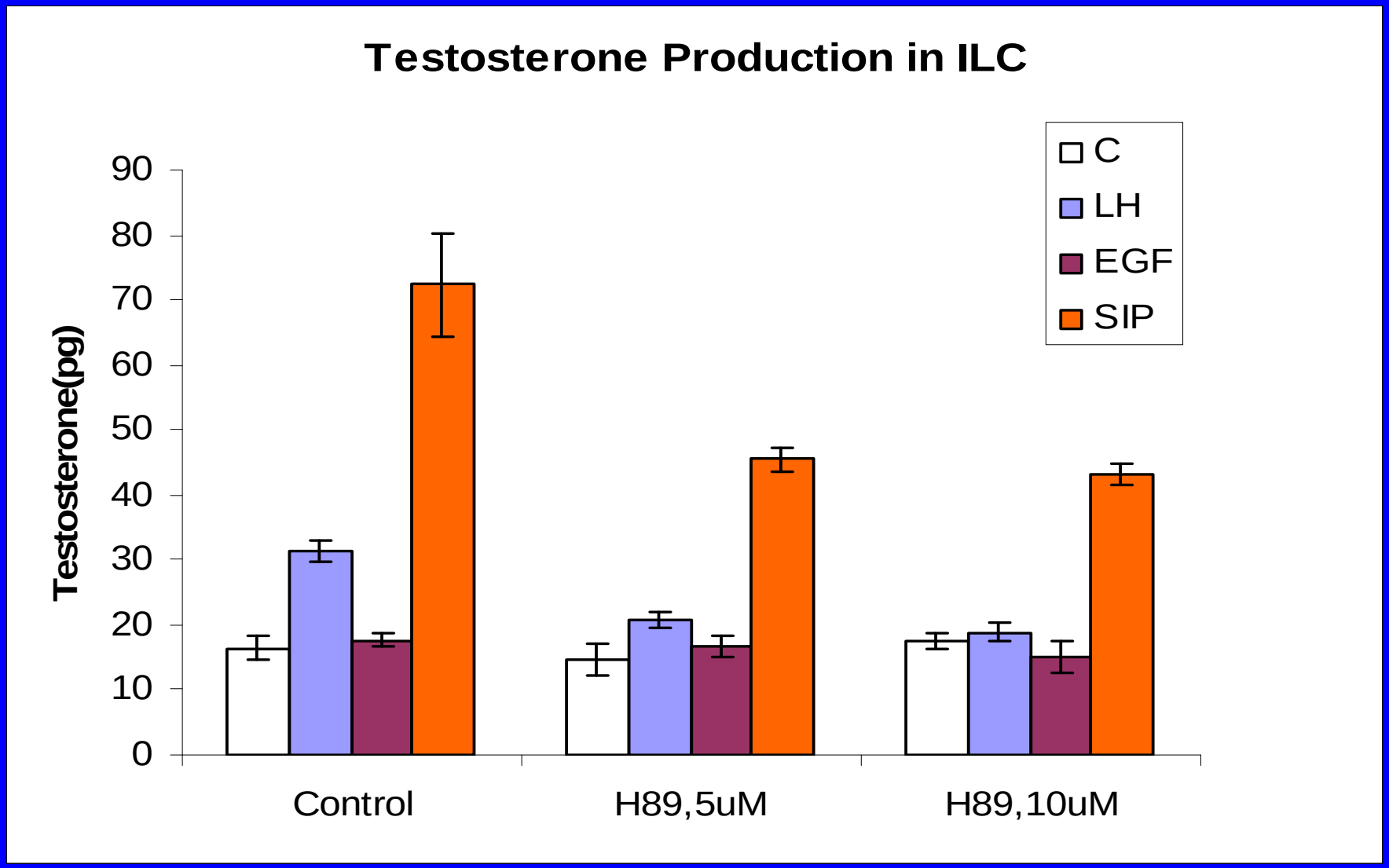
DNA Synthesis



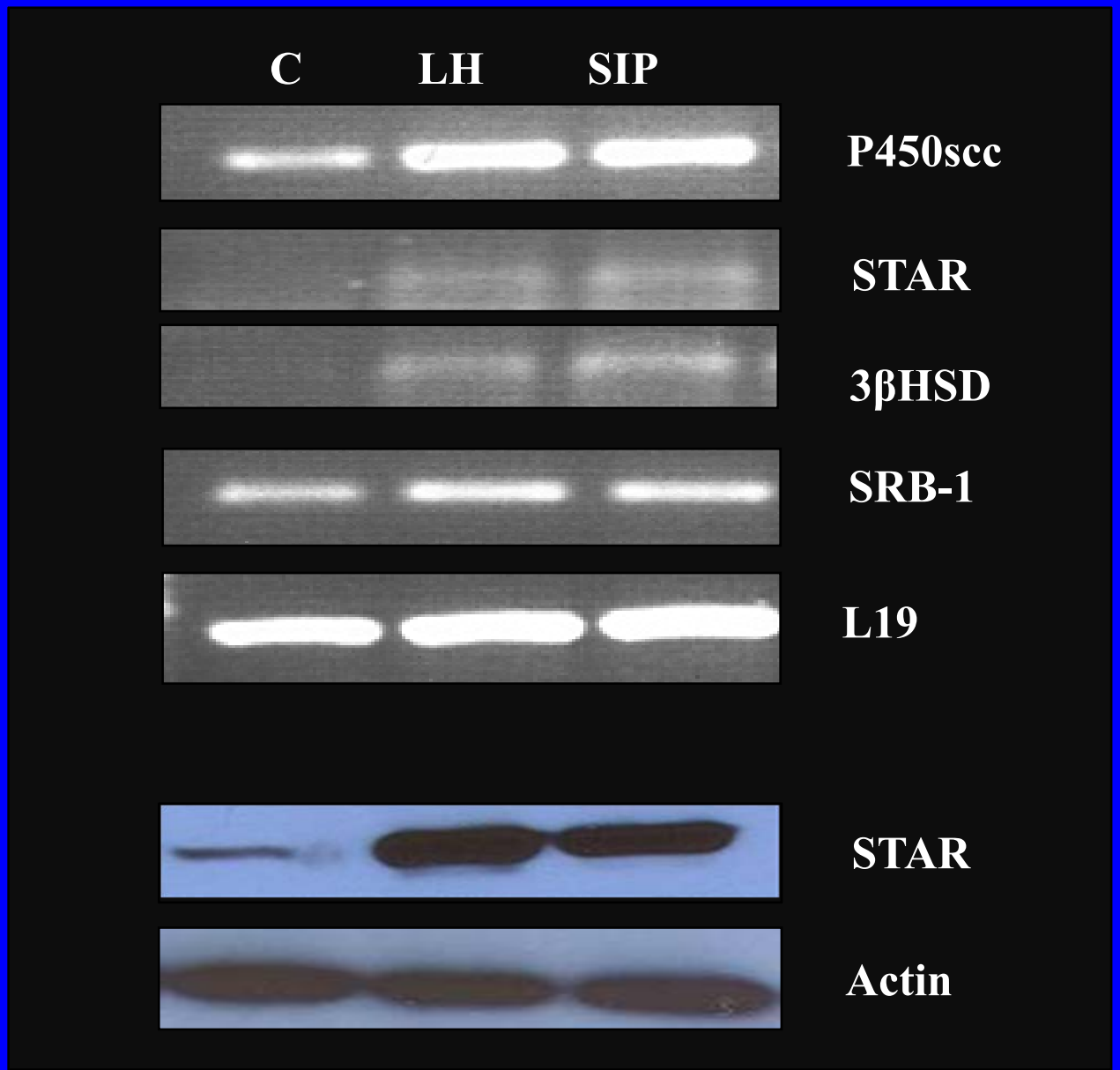
Steroidogenesis



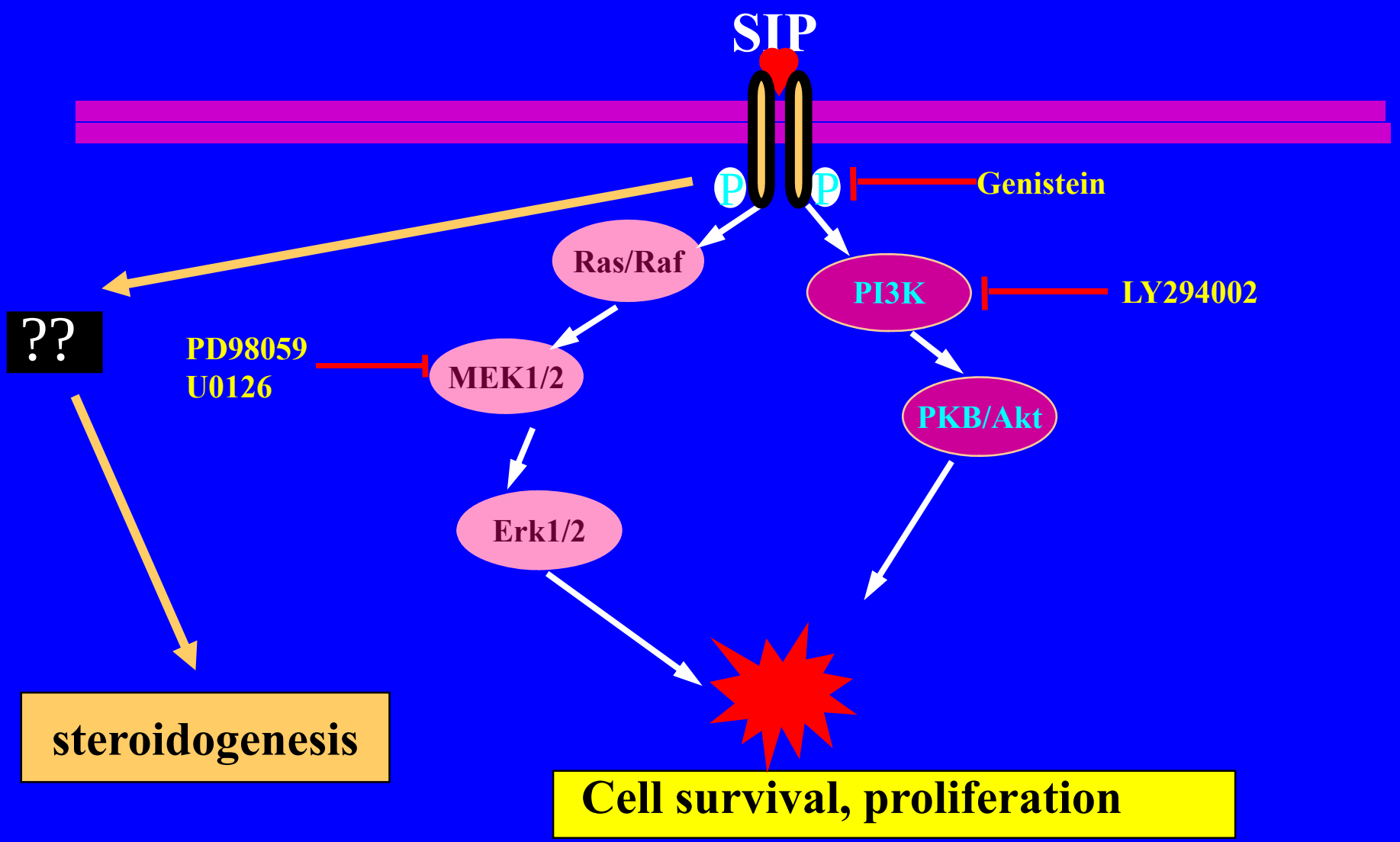
PKA signaling pathway is involved in SIP effects on steroidogenesis in ILC



SIP Effect on Steroidogenic Effectors in ILC



Mechanism of SIP Action



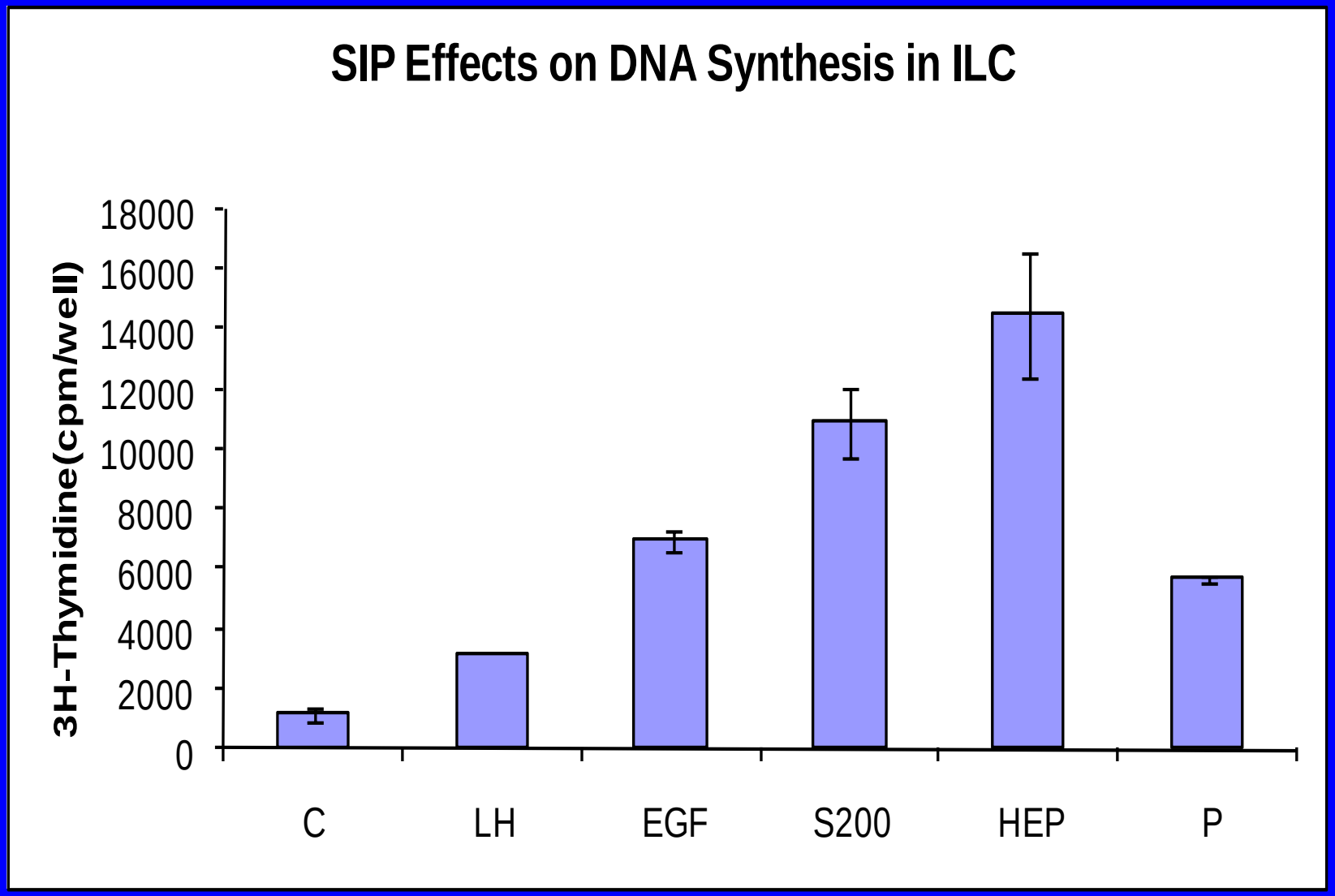
SIP peptide sequences

Peptide 1: **EVQLVESG**

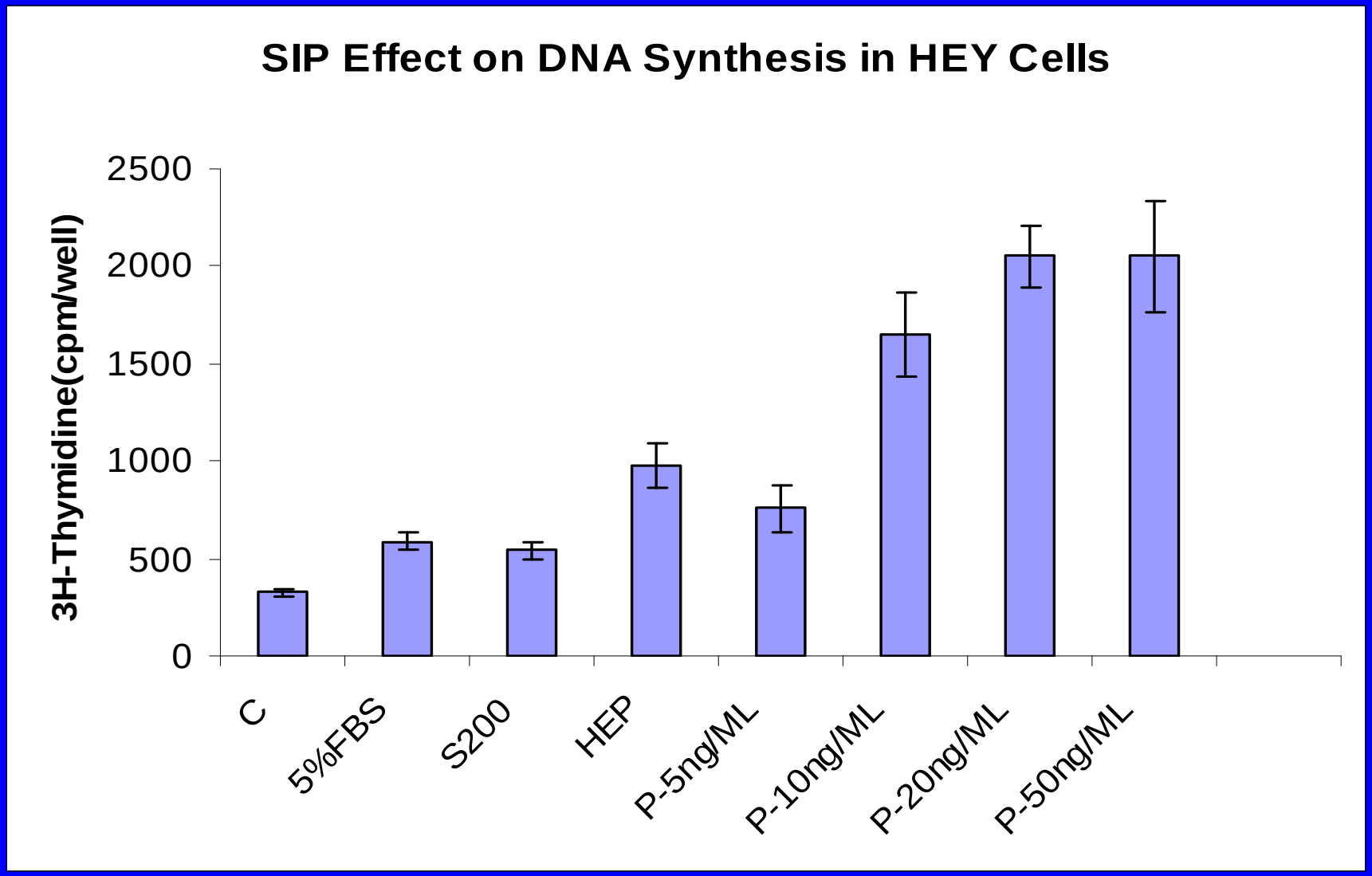
Peptide 2: DVNGGGATLPQPLYQTA

Peptide 3: **FNWYVDGVEVHNAK**

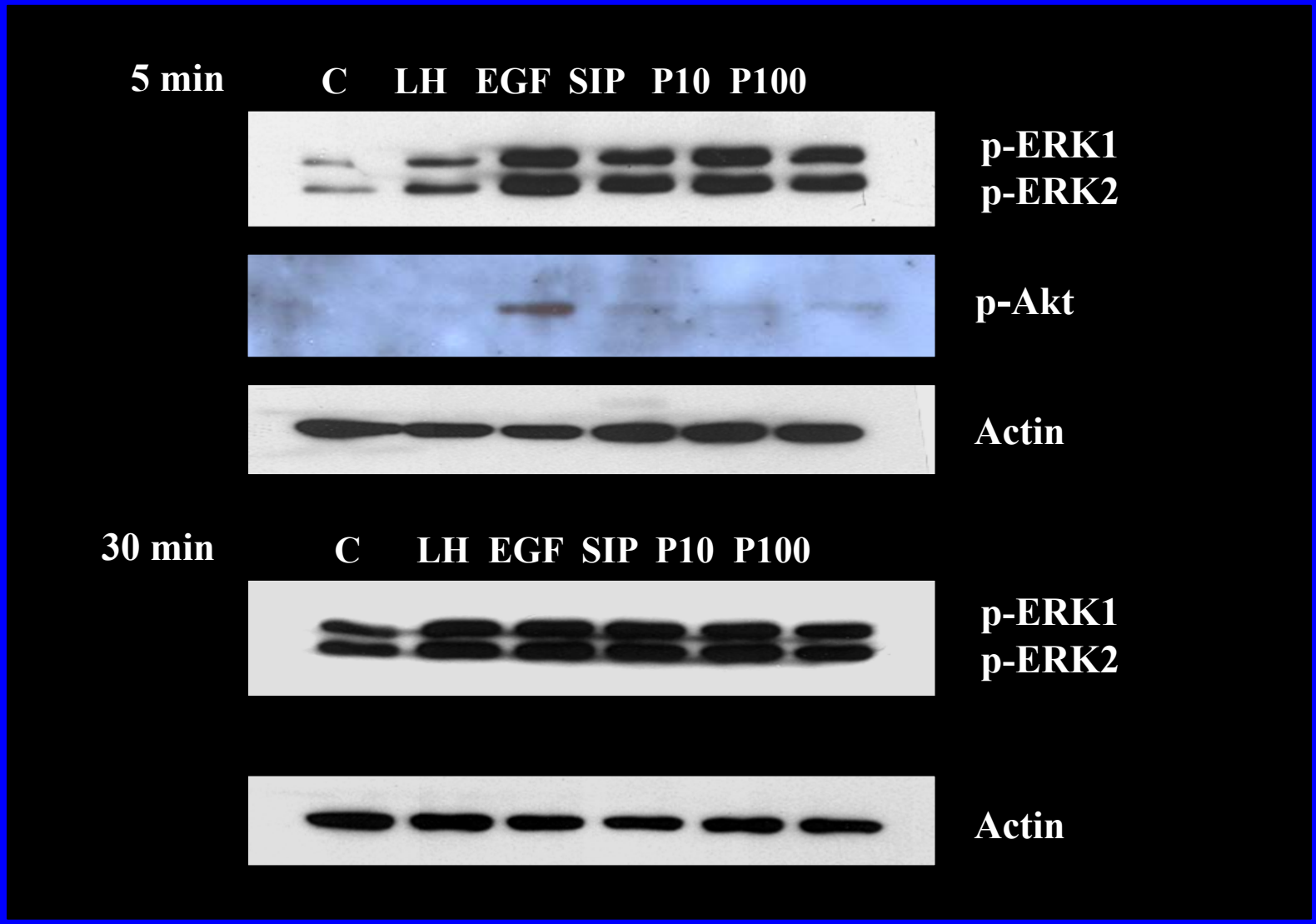
Mitogenic effects of synthetic SIP peptide on in ILC



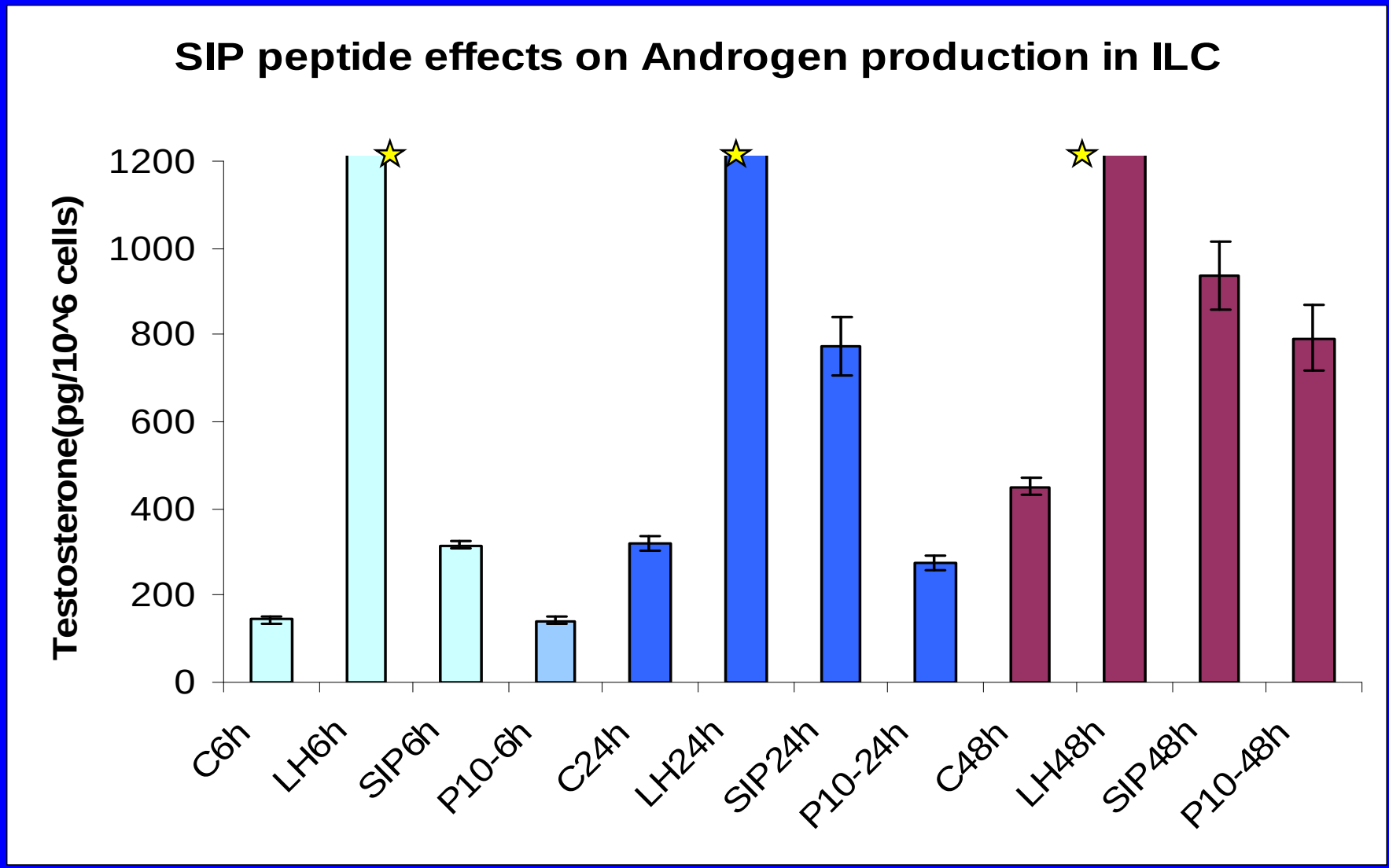
Mitogenic effects of synthetic SIP peptide in HEY cells



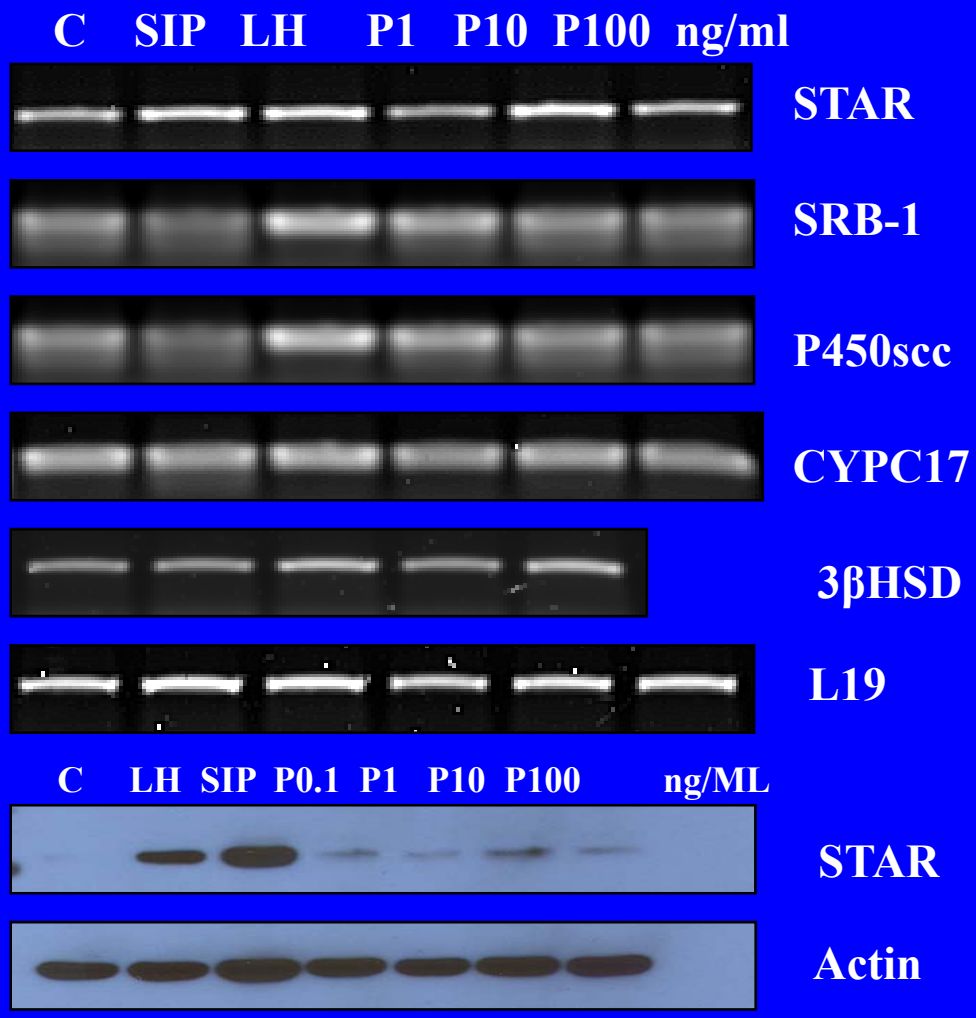
Synthetic SIP peptide activates MAP-kinase and PI3-kinase/Akt pathways in ILC



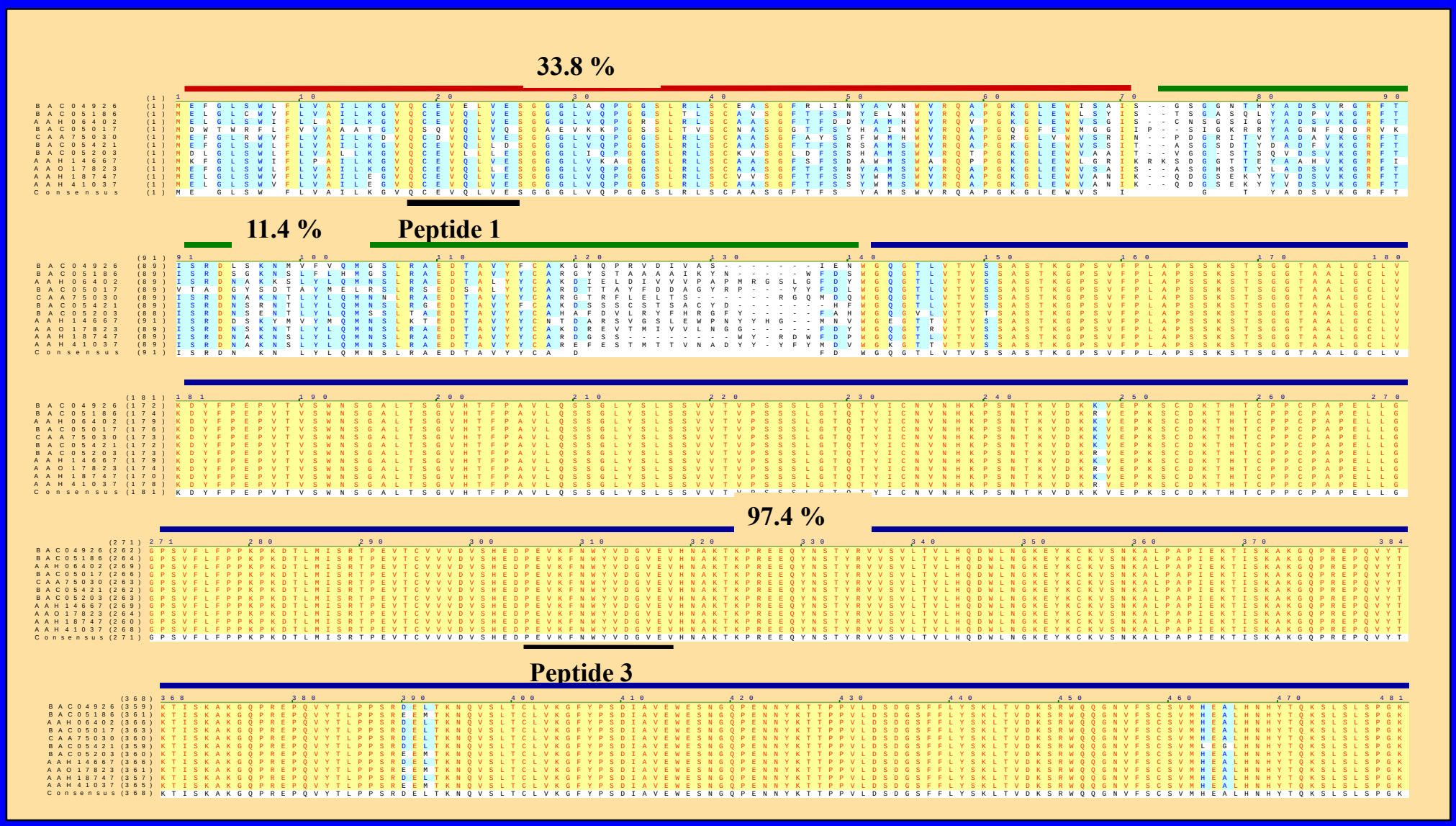
SIP peptide effects on steroidogenesis in ILC



Synthetic SIP peptide Effects on Steroidogenic Effectors in immature rat Leydig cells



Conserved SIP Peptide Sequences Found in Protein Databases



Multiple Sequence Alignment

Summary

- We have isolated and characterized a novel gonadal protein, SIP, which stimulates steroidogenesis and proliferation of gonadal cells.
- SIP belongs to a family of recently recognized human proteins. SIP shows significant homology with a protein isolated from synovial fluid which stimulates proliferation of T-lymphocytes.
- SIP is produced by granulosa cells during normal ovarian cycle and may play a significant physiological role during follicular development, ovulation, luteinization and/or maintenance of luteal function.

Summary (Cont)

- Presence of SIP in ascites fluids from ovarian cancer patients, its secretion by ovarian cancer cells, and its stimulatory effects on proliferation of ovarian epithelial cancer cells suggest that SIP may play a role in the etiology of ovarian cancer.
- SIP is produced by prostate cancer cells and it stimulates proliferation of these cells indicating that SIP is an autocrine growth factor for these cells.
- SIP exerts its effects on target cells via a tyrosine-kinase signaling pathway. SIP effects on cell proliferation are mediated by MAP-kinase and PI3-kinase/Akt pathways.

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